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DISCLAIMER

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INTRODUCTION

Thudichum's suggestion (1884) that mental disorders result from metabolic disturbances[1] was followed by Bleuler's hypothesis (1911) of a metabolic etiology of schizophrenia.[2] Somewhat later, Kallman[3] (1938) and Karlsson[4] (1966) favored the view of a genetic, and presumably metabolic, basis of this disease. The efficacy of mescaline, a methylated compound, in producing a psychotoxicity similar to that seen in schizophrenia led Osmond and Smythies[5] (1952) to propose abnormal transmethylation as a possible cause of disturbances in the psyche. Such hypotheses of a metabolic basis for schizophrenia resulted

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in biochemical studies of this disorder, which particularly involved transmethylation and methylated compounds. Pollin, et al.[6] (1961), and subsequently others[7-10], investigated the adverse effect of L-methionine feeding in the presence of a monoamine oxidase (MAO) inhibitor on some schizophrenic patients. In the presence of an MAO inhibitor, it was anticipated that the catabolism of methylated amines would be inhibited, and that high doses of methionine, the major source of methyl groups for transmethylation, would increase the psychotoxic methylated compounds in the body and result in exacerbation of the schizophrenic condition. Friedhoff's[11] discovery (1962) of dimethoxyphenethylamine (DMPEA), a methylated compound, in the urine of schizophrenics resulted in extensive research on the metabolic origin of this compound. Hoffer[12] (1963) reported clinical improvement of schizophrenics who had been given nicotinic acid or nicotinamide, both of which are methyl group acceptors. Israelstam, et al.[13] (1970), using intravenous ^{14}C-methyl-methionine with $^{14}CO_2$ breath analysis, demonstrated preliminary evidence of altered oxidation of the methionine-methyl carbon in schizophrenic and depressed patients.

The purpose of our study was to investigate further the possible abnormality of methylation in schizophrenia. Using both schizophrenic patients and normal control subjects, this study measured the evolved $^{14}CO_2$ and ^{14}C-protein labeling in leukocytes after incubation with ^{14}C-methyl and ^{14}C-carboxyl methionine.

MATERIALS AND METHODS

Clinical Material

The study included 11 patients with schizophrenia and 7 normal control subjects. All of the patients were under treatment at the time of this study, and had been examined and diagnosed by one of the authors (T. H. M.). The psychiatric diagnoses were in accordance with the International Classification of Diseases[14]. The 7 control subjects were healthy adult volunteers who were not taking any drugs, and who were without history of psychiatric disturbances.

Reagents

1) Heparin: 1,000 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, New York], 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, 5,000 units/ml, and streptomycin, 5,000 ug/ml [GIBCO].

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

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

9) 2M acetate buffer, pH 3.8.

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

11) Scintillation fluid. 2L Toluene, 1L 2-methoxethanol and 16.5g 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.

LEUKOCYTE STUDIES

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

7 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 100g 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 in 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 ml aliquots of the cell suspension in special MEM were transferred into 25 ml Erlenmeyer flasks which contained 2 ml special MEM, and the

flasks closed with an airtight rubber cap. After pre-incubation for 30 min at 37° C in a shaking water bath (50 cycles/min), L-methionine-^{14}C-methyl or L-methionine-^{14}C-carboxyl, 0.5 to 20 uCi, which contained from .054 to .938 u moles/methionine, was injected into each flask. Cell suspensions from each subject were incubated at 1 to 4 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 pre-weighed 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 $^{14}CO_2$, 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 re-weighed, and 4 ml aliquots of the trapping solution were weighed into counting vials. Fifteen ml of scintillation fluid were added to each vial, and ^{14}C activity was quantitated in a liquid scintillation counter using appropriate background and standard samples, quench corrections, and sufficient counts to obtain a standard error of 2.5%.

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

After the $^{14}CO_2$ collection period, the cells were centrifuged at 0^{o} C and washed twice with ice-cold saline. One ml of chilled TCA solution was added and allowed to stand for 30 minutes at 0^{o} 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 ($pM/10^6$ cells/hr) by leukocytes from control subjects and schizophrenic patients are shown in Figure 1. These rates of oxidation are shown for different methionine concentrations in the incubation medium, which range from 17.8 to

to 312 uM/L. Included in Figure 1 is a plot of the least-squares best-fit of each group.

While the rate of methionine-methyl oxidation showed a direct correlation to the methionine concentration in the incubation medium, the rate of methionine-methyl oxidation by the schizophrenic group was less than that of the normal group for each concentration of methionine. While this difference was small at low concentrations, it progressively increased with increasing concentrations of methionine.

The rates of oxidation of the carboxyl carbon of methionine to CO_2 by leukocytes from 2 normal subjects and 2 schizophrenic patients are shown in Table II. These preliminary studies were performed with a concentration of 93.7 u moles/L in the incubation fluid. A relatively wide difference in oxidation of the methyl carbon by the 2 groups has been demonstrated at this concentration (Fig. 1). However, unlike the difference in oxidation of the methyl carbon, no significant difference in oxidation of the carboxyl carbon of methionine was noted in the leukocytes of either the control subjects or the schizophrenic patients.

The incorporation of ^{14}C-methyl carbon into protein (and other TCA precipitable material) by leukocytes as a function of methionine concentration of incubating media is shown in Figure 2 for both control subjects and schizophrenic patients. While a progressive increase in incorporation is noted with an increasing concentration of methionine, no significant difference is noted between the experimental groups.

DISCUSSION

The studies of mescaline effect[5], methionine and MAO-inhibitor exacerbation[6-10], and nicotinamide improvement[12] of some schizophrenics, the finding of DMPEA in the urine of some schizophrenics[11], and the altered in vivo oxidation of methionine-methyl carbon in the schizophrenic patient[13], all have suggested the possibility that altered methylation may be associated with the schizophrenic state. It has been postulated that such a metabolic abnormality would result in the production of abnormal amounts and/or kinds of psychoactive methylated amines which, in turn, are involved in producing schizophrenia. Since the methyl carbon of methionine is the major metabolic source of methyl groups for transmethylation[15, 16], the possible biochemical abnormality associated with schizophrenia may be clarified by tracing its metabolic fate in the schizophrenic patient. Leukocytes were selected for this study because of their possession of the requisite cell organelles, their easy availability, and because they can be utilized readily in a variety of short-term in vitro biochemical studies, including those involving ^{14}C-labeled substrates[17, 18].

The metabolic fate of the methyl carbon of methionine is manifold[15, 16]. It can appear in methylated compounds, in CO_2, and in proteins through incorporation of methionine and other amino acids which subsequently contain methionine-methyl carbon atoms. The decreased oxidation of the methyl-carbon of methionine which was noted in the leukocytes of schizophrenic patients could be explained by any of the following: (a) decreased transmem-

brane passage of methionine into cells or intracellular organelles, (b) a derangement in the enzyme(s) or metabolic pool(s) involved with the oxidation of methionine or its methyl group to CO_2, (c) increased diversion of the methyl group into transmethylation processes, and (d) increased incorporation into protein.

The progressive increase of the difference in rates of methyl carbon oxidation caused by increasing the methionine concentration of the incubation medium, and the similar oxidation of the carboxyl-carbon of methionine by both groups, exclude differences in methionine pool size, membrane transport, and metabolism of methionine other than its methyl carbon, as explanations for the decreased rate of oxidation of methionine-methyl carbon in the schizophrenic group. The lack of altered methyl-carbon incorporation into protein by the leukocytes of schizophrenics suggests that changes in the rate of protein synthesis and/or the rate of methionine-methyl incorporation into protein are not responsible for the decreased oxidation of methionine-methyl carbon in this disorder. The decreased rate of methionine-methyl carbon oxidation in leukocytes of schizophrenics could be explained by either an increased diversion of methyl groups into transmethylation process, or an alteration in the enzymatic pathway or metabolic pools that are involved with the oxidation of a methyl group after its removal from the methionine molecule.

Although drug effect cannot be absolutely excluded as a cause of the decreased oxidation of the methionine-methyl carbon by the leukocytes of schizophrenics, the lack of any consistent difference in methyl-carbon oxi-

dation between those 3 patients who did not receive drugs and the 8 patients who received a variety of drugs suggests that the medications administered to the study group probably did not play a role in producing the observed phenomenon.

These initial findings of altered methionine-methyl oxidation in the leukocytes of schizophrenic patients suggest an abnormality in transmethylation 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 it may be systemic in nature. If these preliminary findings are confirmed by further studies, they not only will support the hypothesis of a biochemical basis for schizophrenia, but also will suggest possible new approaches to the study of this disease.

SUMMARY

The purpose of this study was to investigate further the possible abnormality of methyl-carbon metabolism in schizophrenics. Leukocytes from 7 normal subjects and 11 schizophrenic patients were incubated with ^{14}C-methyl methionine, and the evolved $^{14}CO_2$ and ^{14}C-leukocyte protein labeling was measured. The rate of oxidation of methionine-methyl carbon to CO_2 by leukocytes of schizophrenic patients was less than that noted in leukocytes of normal subjects. While this difference was small at low concentrations of methionine in the incubation medium, it progressively increased with increasing concentration of methionine. No differences in ^{14}C-protein labeling was noted between the two study groups. Preliminary studies using ^{14}C-

carboxyl-methionine revealed no difference in oxidation of methionine-carboxyl carbon to CO_2 by the two groups. These findings suggest that the decreased rate of methionine-methyl carbon oxidation in leukocytes of schizophrenics could be explained by either an increase diversion of methyl group into trans-methylation processes, or an alteration in the enzymatic pathway or metabolic pools that are involved with the oxidation of methyl group after its removal from the methionine molecule.

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TABLE I

CLINICAL AND THERAPEUTIC DATA

DIAGNOSIS	CLINICAL MANIFESTATIONS							DRUG THERAPY	
	Age	Sex	Duration of Illness (Years)	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 150 mg 300
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 Phenobarbital Trihexyphenidyl Hydrochloride Diphenhydramine	75 mg 6 mg 100 mg 6 mg 150 mg
6. Residual Schizophrenic	36	M	>14	+	+	+	+	Niacinamide	1 mg
7. Chronic Undifferentiated	26	F	5	+	+	+	+	None	
8. Residual Schizophrenic	33	M	15	0	0	+	+	None	
9. Schizo-Affective Circular	26	M	20	0	+	+	+	Trifluoperazine Hydrochloride Diazepam	4 mg 15 mg
10. Residual Schizophrenic	47	F	>10	0	0	0	0	None	
11. Chronic Undifferentiated	22	F	3	+	+	+	0	Haloperidol Diazepam	4 mg 15 mg

+ Present
0 None
? Unknown

TABLE II

OXIDATION OF THE CARBOXYL CARBON OF METHIONINE*
BY LEUKOCYTES FROM
CONTROL AND SCHIZOPHRENIC PATIENTS

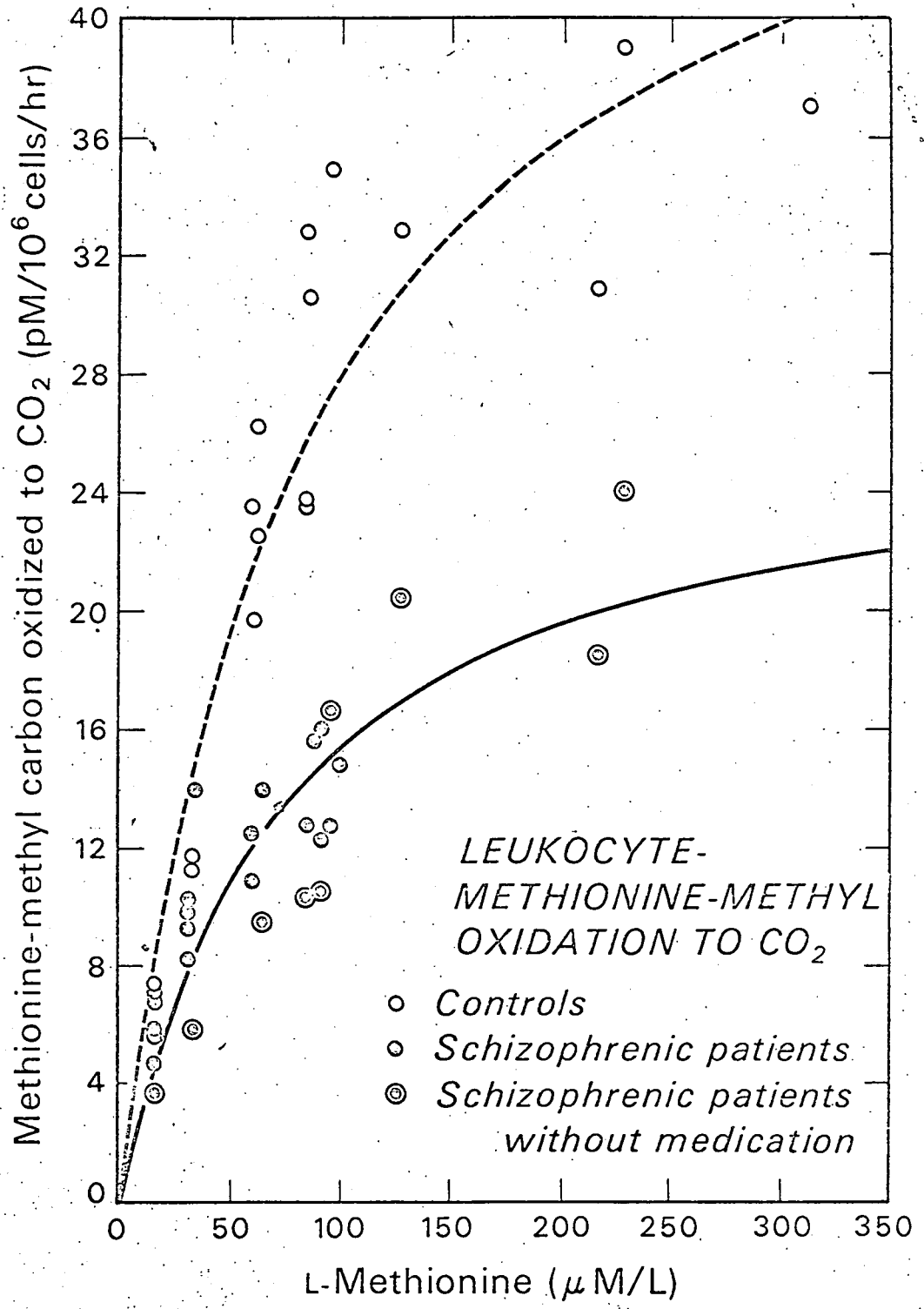
Subject	Oxidation of Carboxyl-Carbon of Methionine pM/10^6 cells/hr
Control	12.3
Control	12.1
Schizophrenic	12.0
Schizophrenic	12.1

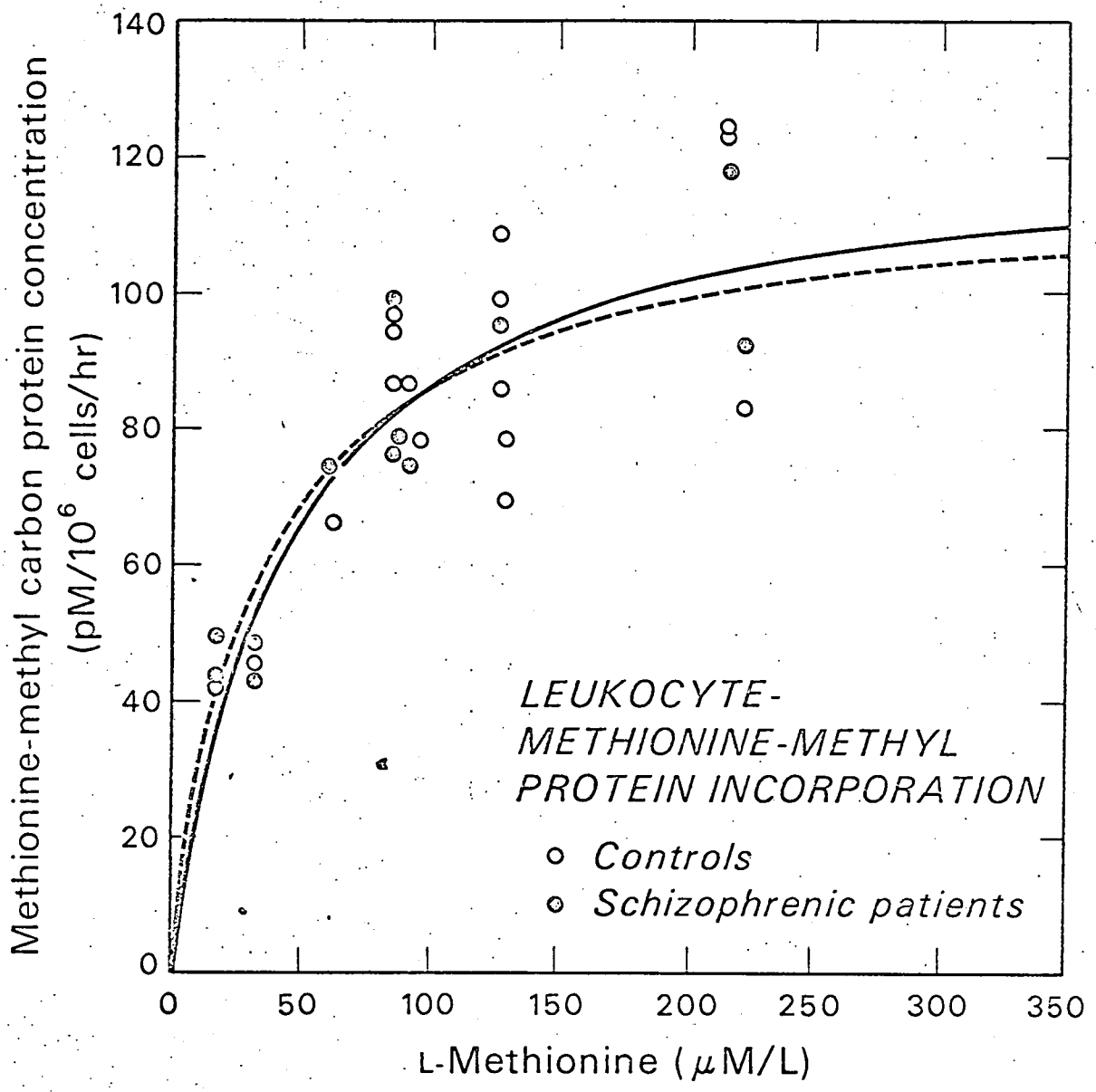
* Methionine concentration in incubation fluid of 93.7 u moles/L.

LEGENDS

FIG. 1 The rates of oxidation of methionine-methyl carbon to CO_2 ($pM/10^6$ cells/hr) by leukocytes from control subjects o , schizophrenic patients ⊙ , and schizophrenic patients without medication ◎ , as a function of the concentration of methionine (u moles/ L) in the incubation medium.

FIG. 2 The rate of incorporation into protein of methionine-methyl carbon ($pM/10^6$ cells/hr) by leukocytes from control subjects o and schizophrenic patients ⊙ as a function of the concentration of methionine (u moles/ L) in the incubation medium.


Methionine-methyl carbon oxidized to CO_2 (pM/10^6cells/hr)
0
4
8
12
16
20
24
28
32
36
40
0
50
100
150
200
250
300
350
L-Methionine (μM/L)
LEUKOCYTE-METHIONINE-METHYL OXIDATION TO CO_2
Controls
Schizophrenic patients
Schizophrenic patients without medication


Methionine-methyl carbon protein concentration
(pM/10^6 cells/hr)
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
350
L-Methionine (μM/L)
LEUKOCYTE-
METHIONINE-METHYL
PROTEIN INCORPORATION
Controls
Schizophrenic patients

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26 October 1972

Dr. Ernest L. Dobson
Biomedical Research Division
Lawrence Radiation Laboratory
Berkeley, California 94720

Dear Dr. Dobson:

I'm happy to let you know that the "Alteration in Methionine Metabolism of Leukocytes in Schizophrenia" manuscript is finished, and that I have submitted our paper to the Journal of Psychiatric Research.

Enclosed, for your interest, is a copy of the manuscript.

Sincerely yours,

Mathews B. Fish, M.D.
Associate Director
Clinical Laboratories

MBF:sl

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26 October 1972

Seymour S. Kety, M.D.
Editor-in-Chief
Journal of Psychiatric Research
Psychiatry Research Laboratories
Research-4
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Boston, Massachusetts 02114

Dear Dr. Kety:

We would like to submit the enclosed manuscript, "Alteration in Methionine Metabolism of Leukocytes in Schizophrenia", to be considered for publication in the JOURNAL OF PSYCHIATRIC RESEARCH.

You will find here the original and one copy of the manuscript. Thank you for your consideration.

Sincerely yours,

Mathews B. Fish, M.D.
Associate Director
Clinical Laboratories
San Francisco General Hospital

Associate Professor
Department of Clinical Pathology
and Laboratory Medicine
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