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Folder No. <u>FIELD TASK PROPOSAL/AGREEMENT</u>	
Notes <u>LDDamphetamine 133-1</u>	
Found By <u>Eden Holmes</u>	
Date <u>1987</u>	

11-D methyl studies in schizophrenia

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(4-80)

U.S. DEPARTMENT OF ENERGY
FIELD TASK PROPOSAL/AGREEMENT

1. WORK PACKAGE NUMBER	2. TASK NO.	3. REV. NO. 0	4. PROJECT NO.	5. DATE PREPARED 04/01/82	6. CONTRACTOR NUMBER 4458/003646
7. TASK TITLE Metabolism in Brain Disorders				8. WORK PACKAGE TITLE	
9. BUDGET AND REPORTING CODE HB 02 01 000		10. TASK TERM Begin: Continuing End: Open		11. CONTRACTOR NAME University of California Lawrence Berkeley Laboratory	12. CODE (see instructions) 999
13. CONTRACTOR TASK MANAGER (Name: Last, First, MI) (FTS No.) Alpen, Edward L. 451-5206				14. PRINCIPAL INVESTIGATORS (Name: Last, First, MI) Sargent, Thornton III	
15. WORK LOCATION (See instructions): Name of facility, City, State, Zip Code Lawrence Berkeley Laboratory Berkeley, California 94720				16. Is this task included in the Institutional Plan? <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	17. Does this task include any management services efforts? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
18. TASK DESCRIPTION (Approach, relation to work package, in 200 words or less)					

In this task we are developing radioactive tracer techniques to study schizophrenia in human patients. A new iodoamphetamine is being synthesized which will use 3.6 min ¹²²I from a 20 hr ¹²²Xe generator, and measure regional cerebral blood flow with PET images of brain. We will attempt to verify reports of other workers of abnormal brain metabolic rate in schizophrenia. [¹¹C-methyl]-L-methionine has been synthesized and will be used in human studies to measure body organ kinetics of methyl metabolism, first using the Anger whole-body scanner and then the PET ring modified for whole-body scanning. The PET ring will then be used for detailed dynamic pictures of a single plane through the limbic system of the brain. Simultaneously during all scans, ¹¹CO₂ respirometry will measure methyl oxidation, and plasma samples will be taken for HPLC analysis of metabolites. All measurements will be incorporated into a compartment model system to identify and quantify the abnormal methyl metabolism in schizophrenia found in our earlier work. Demonstration of a biochemical abnormality would speed a cure for this widespread disease.

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(Signature)		(Date)	
20. DETAIL ATTACHMENTS: (See instructions)			
<input type="checkbox"/> a. Facility Requirements	<input checked="" type="checkbox"/> d. Background	<input checked="" type="checkbox"/> g. Future accomplishments	<input type="checkbox"/> i. Explanation of milestones
<input checked="" type="checkbox"/> b. Instructions	<input checked="" type="checkbox"/> h. Approach	<input checked="" type="checkbox"/> n. Relationships to other projects	<input type="checkbox"/> k. ZBB Detail
<input checked="" type="checkbox"/> c. Budget	<input checked="" type="checkbox"/> l. Technical progress	<input type="checkbox"/> o. Environmental assessment	<input checked="" type="checkbox"/> t. Other (Specify):

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PRINCIPAL INVESTIGATOR Sargent, Thornton, III	DATE PREPARED 04/01/82	TASK NUMBER	REVISION NUMBER 0

20. DETAIL ATTACHMENTS:

Attachment f. Technical Progress:(continued)

basis of methylation reactions which are required in excess by malignant cells for their multiplication. This is a large field of research in itself, and work in this project may impinge upon it in future years.

Attachment g. Future Accomplishments:

In FY 83 we will have the $^{122}\text{IDNNA}$ system operational using the optimal chemical form of the radiopharmaceutical as determined in experiments during FY 82 as described in attachment e. We will begin studies to compare rCBF, as measured with $^{122}\text{IDNNA}$, to rCMF as measured by ^{18}FDG . The ^{18}FDG studies have already begun in FY 82 in patients with Alzheimer's dementia in our collaborative studies with Dr. Friedland at the Martinez VA and will begin with schizophrenic patients from PAVAMC in late FY 82. We expect to be able to answer the question definitively during FY 83 as to whether there are specific regional differences in energy metabolism and blood flow in schizophrenia.

We will begin studies with ^{11}C -methionine in patients and normals, using the whole-body scanner. Dr. Huesman, in project 4454/000331 is developing a system by which the PET ring can be used as a whole-body tomographic scanner, and as soon as this is operational it will be used instead of the existing Anger Mark II Scanner. $^{11}\text{CO}_2$ respirometry studies will be done simultaneously; the appropriate extensions to connect the breath collection helmet from the scanner bed to the respirometer are in place. HPLC methods for separation of at least some of the metabolites we wish to study will also be developed at this time and blood sampling will be done in conjunction with each patient study.

During FY 83 we will continue to perfect our HPLC plasma separation techniques to be able to identify and quantify a greater number of methyl metabolites. Investigation of metabolites by administering ^{11}C methionine to leukocyte cultures will then be initiated because of the grant sensitivity of the method, and the possibility of measuring intracellular metabolism of cells from schizophrenic patients. Methods for ^{11}C synthesis of other metabolites in the methyl chain will be studied for future tracers to be applied in PET studies for more detailed investigation of methyl metabolism. The method we have devised to synthesize [^{11}C -beta] tyrosine will be pursued to provide a labelled catecholamine for brain PET studies.

In FY 84 we expect to have established a picture of methionine kinetics in normals and schizophrenics in terms of whole-body metabolism, and will begin complete coordinated studies with the PET system. If the 4-ring, 7 plane system being designed and built by Dr. Derenzo in project 4450/002420 is operational, it will be employed to provide simultaneous brain and liver tomographs, as the rings will be separable. The coordinated studies will consist of: a CT scan to provide the correct alignment for the desired image plane; a $^{122}\text{IDNNA}$ study to provide quantitation regional rCBF; injection of ^{11}C -methionine followed by simultaneous measures of regional brain ^{11}C distribution; $^{11}\text{CO}_2$ respirometry and blood sampling for plasma HPLC analysis. All of this data will be incorporated in a compartment model analysis to provide an overall picture of methyl metabolism and its differences, which we expect to find in patients with schizophrenia. We will be investigating intracellular methyl metabolism by ^{11}C methionine labelling of leukocytes with HPLC analysis. ^{11}C labelling methods for other metabolites, such as sarcosine and tyrosine, will be in

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20. DETAIL ATTACHMENTS:

Attachment g. Future Accomplishments: (continued)

progress and these labelled molecules will be staged for human studies.

Attachment h. Relationships to Other Projects:

This project is closely related and dependent upon other projects in the Research Medicine Group. Synthesis of ¹¹C-methionine and development of the ¹²²IDNNA system is being done in collaboration with Yukio Yano's Experimental Medicine Development Group (4452-000330). The PET system, development of the whole-body scan tomograph system, and compartment modelling is being done principally by Drs. Budinger, Huesman and Derenzo in the Experimental Medicine Clinical group. 4454/000331.

Development of the 4 ring 7 plane PET system which will eventually be used in this program is being done by Dr. Derenzo's Positron 3/D Imaging Instrument program, 4450/002420.

Attachment i. Environmental Assessment: N/A

Attachment j. Explanation of Milestones: N/A

Attachment k. ZBB Detail: N/A

Attachment l. Equipment FY 83:

Gamma counter for radioactive peak measurement from HPLC output. Special fabrication	\$9,000.
High Performance Liquid Chromatography system to provide second parallel track of analysis.	\$9,000.
High speed air driven microcentrifuge, for rapid separation of blood samples.	\$1,200.

FY 84

High Performance Liquid Chromatography System. Required for third parallel track to provide rapid separation of labelled metabolites in different solvent separation systems.	\$10,000
Gamma counter for HPLC output.	\$9,000

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(Date)

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| <input checked="" type="checkbox"/> b. Publications | <input checked="" type="checkbox"/> e. Approach | <input checked="" type="checkbox"/> h. Relationships to other projects | <input type="checkbox"/> k. ZBB Detail |
| <input checked="" type="checkbox"/> c. Budget | <input checked="" type="checkbox"/> f. Technical progress | <input type="checkbox"/> i. Environmental assessment | <input checked="" type="checkbox"/> l. Other (Specify): |

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PRINCIPAL INVESTIGATOR Sargent, Thornton III	DATE PREPARED 04/01/82	TASK NUMBER	REVISION NUMBER 0

20. DETAIL ATTACHMENTS:

Attachment b. Publications

Bustany, P., Sargent, T., Saudubray, J.M., Henry, J.F. and Comar, D. Regional human brain uptake and protein incorporation of ^{14}C -L-Methionine studied in vivo with PET. J. Cerebral Blood Flow and Metabol. 1: suppl. 1, S 17-18 (1981).

Submitted:

Sargent III, T., Shulgin, A.T., Mathis, C. and Budinger, T.F. A new iodo-amphetamine for rapid positron tomographic measurement of brain blood flow with ^{122}I . Submitted to: 3rd World Congress of Nuclear Medicine, Paris Aug. 29-Sept. 2 '82.

Planned Publications:

Sargent III, T., Braun, U., Braun, G., Kusubov, N., and Bristol, K.S. Cerebral and peripheral demethylation of phenolic and indolic psychotomimetics measured by expired $^{14}\text{CO}_2$: To be submitted to Neuropsychobiology.

Sargent III, T. and Bristol, K.S. Oxidation of the one carbon pool studied by injection of labelled intermediates and measurement of expired $^{14}\text{CO}_2$ in rats. To be submitted to J. Nutrition.

Sargent III, T. and Kusubov N. Effects of dietary methionine on oxidation of the ^{14}C labeled methyl carbon studied in rats and humans by measuring expired $^{14}\text{CO}_2$. To be submitted to: J. Nutrition.

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20. DETAIL ATTACHMENTS:
Attachment c. Purpose:

Mental illness is one of the most common and debilitating afflictions, affecting its victims and their families throughout long portions of their lifetimes, yet is one of the most poorly studied of all public health problems in terms of basic chemical and physical science. Over 10% of the U.S. population is suffering from one or the other of these mental disorders: schizophrenia (1%), manic-depressive affective disorders (2%), and senile dementia (8%). The major psychoses, schizophrenia and affective disorders, are now widely accepted to be genetically determined illnesses affecting the central nervous system, and many laboratories including ours have narrowed the search for the biochemical basis to certain areas of brain metabolism. Because these illnesses are unique to the human species, animal studies provide limited information. It is for this reason that the instrumentation developed at this laboratory for study in human patients of quantitative in vivo biochemistry by use of radioisotopes is uniquely valuable.

The Donner 280 crystal positron emission tomograph (PET) system will be used to determine kinetics of [¹¹C-methyl]-L-methionine and an ¹²²I flow indicator in specific brain regions; the Mark II whole body scanner will measure brain and liver differences; ¹¹CO₂ respirometry will measure global oxidative kinetics; and High Performance Liquid Chromatography (HPLC) will be used to measure specific metabolite kinetics in plasma. We have and are developing special synthetic methods for short-lived positron isotope labelling including [¹¹C-methyl]-L-methionine, ¹²²I amphetamines for brain blood flow measurements and ¹¹C tyrosine. This combination of in vivo radioisotope techniques for study of mental illness is uniquely available at this laboratory, and has the potential of providing new insight into the biochemical causes of these diseases and thus of devising more successful treatment.

Attachment d. Background:

Mental illness is one of the most distressing of man's ailments, and at the same time has been one of the most resistant to scientific investigation because of the difficulty of investigating human brain function noninvasively. A number of recent advances have opened up new methods for such investigations, and among these the use of radioisotope tracer technology with PET and HPLC analysis offer possibilities of quantitative measures of dynamic biochemical function of the brain. It is these techniques, to which we have contributed and developed in prior work, which we propose to utilize to investigate human mental disease. There are two principal overlapping areas on which we will concentrate: 1) measurement of brain blood flow and regional cerebral metabolic rate (rCMR) for glucose, and 2) quantitation of biochemical abnormalities in transmethylation and catecholamine pathways.

1. Regional cerebral blood flow (rCBF) has become of increasing interest recently because this measurement may be of importance in many brain disorders in addition to mental disease. We propose to increase our attention to this area because a) our prior work has been the basis for recent development of radiopharmaceuticals for rCBF determination by ourselves and others, and we are in a unique position to make significant advances in this field, b) there have been a number of reports of rCBF abnormalities in mental disorders, and c) a measure of rCBF will be important to our measurements of ¹¹C-methyl transmethylation kinetics.

The first halogenated amphetamine shown to concentrate in brain, 4-bromo-2,5-dimethoxyphenylisopropylamine (4-BrDPIA; Fig. 1, compd a) was reported by us in 1975 (1,2,3) using ⁸²Br and ⁷⁷Br and the Anger Mark II whole body scanner. This

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Attachment d. Background (continued):

was followed by our report of the brain uptake of the analogous compound made with ^{123}I (Fig. 1, compd b) in 1978 (4,5,6) which included the first single photon tomographic reconstruction brain scan (6). The extraction of the ^{123}I analog in monkey brain measured with an Anger camera appeared to be a single-pass uptake phenomenon, with the activity remaining constant after the first minute, an ideal situation for rCBF measurement if the agent indeed measures flow. Following this idea, Winchell et al. (7,8) studied a large series of compounds and chose the 4-iodo-N-isopropyl amphetamine (IAMPH, Fig. 1, compd c) to pursue clinical studies. This compound has been studied by Kuhl and his co-workers at UCLA and has been shown by comparison with labelled microspheres to be an accurate indicator of regional flow (47). It has been used in over 100 patients by Dr. Thomas Hill at Boston Deaconess Hospital, in which excellent flow images have been obtained by single photon tomography. A recent report has shown decreased uptake in brain tumors compared to normal brain (37). Diamine congeners (HIPO) being investigated by Kung and Blau appear to give comparable results (9). 4-IAMPH and the diamine compounds appear to be of value in clinical situations in which quantitatively accurate, attenuation corrected single photon tomography is available, and will be useful in clinical situations with an Anger camera even though only qualitative images will be possible. The recent and future potential of single photon techniques has just been reviewed and discussed by Budinger (34). However, there are several conditions that cannot be met by these after two types of compounds: a) labelling is by exchange reaction, which inherently is slow and is not capable of yielding very high specific activity, b) the isotope has a relatively long half-life, resulting in the inability to perform repeat studies in rapid succession and thereby excluding the possibility of assessing the response to manipulation or stimulation. In addition, the patient dose is larger than need be; c) ^{123}I is not a positron emitter and thus cannot be used in the quantitative PET systems; the only positron emitters of iodine available are ^{124}I , with too long a half life (4 days), and ^{124}I , with a half life too short for exchange labelling. When performing rCBF measurements to be used in conjunction with subsequent brain studies in a PET system, it is essential that the data be also obtained by PET so that the data will be consistent (in the same format and measurement modality).

Compounds of the type that we have developed, with the 2,5 dimethoxy substitution on the ring (Fig. 1, compds e and f), have the potential of overcoming the problems outlined above; the details of the system we propose to develop will be outlined under attachments e. and f.

Our Research Medicine Group has also developed methodologies for measuring other parameters of cerebral vascular function, which may also find application in this project under attachments e. and f. [cf. project 4454/000331]. ^{82}Rb has been used to quantitatively measure heart perfusion (10) and its use for measurement of brain blood volume (11) and permeability (12) have also been reported.

It is generally considered that under normal conditions rCBF is coupled to regional cerebral metabolite rate (rCMR). Some recent work has reported that rCMR as measured by ^{18}F -deoxyglucose (^{18}F FDG) is altered in schizophrenia (38). The ^{14}C deoxyglucose method for study of brain regional glucose metabolism, originally developed by Sokoloff et al. (51), utilizes trapping of the unmetabolizable phosphorylated product ^{14}C -deoxyglucose-6-phosphate to quantitate glucose uptake autoradiographically. This method has been extended by the Brookhaven group under Wolff, and the UCLA group under Kuhl, by labelling deoxyglucose with ^{18}F (^{18}F FDG) and performing in vivo autoradiography by PET scanning. Their preliminary studies have indicated decreased frontal

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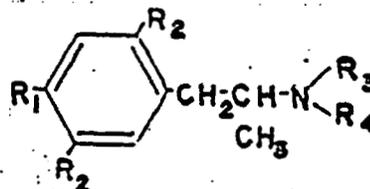
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20. DETAIL ATTACHMENTS:

Attachment d. Background (continued):



Compound	R ₁	R ₂	R ₃	R ₄
a	Br	OCH ₃	H	H
b	I	OCH ₃	H	H
c	I	H	CH(CH ₃) ₂	H
d	H	OCH ₃	CH ₃	CH ₃
e	I	OCH ₃	CH ₃	CH ₃
f	I	OCH ₃	CH ₂ CH ₃	CH ₂ CH ₃

Figure 1. Structures of various substituted amphetamine compounds referred to in the text.

lobe uptake in schizophrenia (38), unilaterally decreased uptake in manic-depressives, and decreased localized white matter uptake in senile dementia, the latter comparable to that found with ¹¹C-methionine during Dr. Sargent's year at Dominique Comar's laboratory in Orsay, France. The ¹⁸F-DG method is a good tool for measuring localized differences in glucose metabolism of the brain, but does not give an indication of the specific causes of such differences; we are developing more specifically labelled metabolic intermediates in order to identify the biochemical lesion.

However, the FDG method has serious drawbacks. The examination requires at least 40 min after injection; thus, it is not certain when and if changes in sensory stimulation affects regional uptake, and it is difficult to maintain a constant stimulation during this entire period, especially with schizophrenics. While relative uptake rates in different areas of the same brain can be measured, to obtain absolute values, arterial or "arterialized" blood must be obtained for the entire period and the "lumped constant" and the rate constants must be similar for all subjects. When we have developed our new iodo-amphetamine rCBF agent we will be able to measure in the same patient, rCBF and rCMR, using ¹⁸F-DG as will be described in attachment e.

2. Transmethylation and catecholamine pathways. The currently most popular theories for the biochemical basis of schizophrenia are the dopamine hypothesis, in which a hyperactivity of catecholaminergic neurosis is hypothesized, and the transmethylation hypothesis. Our principal interest is on the study of abnormal transmethylation reactions. It should be noted at the outset that we are considering this hypothesis with a focus

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Attachment d. Background (continued):

which is not identical with that usually associated with the transmethylation theory. The original hypothesis (14) proposed that schizophrenia is caused by endogenous production of an abnormally methylated metabolite resembling mescaline which causes psychotic phenomena of similar form to those of the psychotomimetic drugs. Our present working hypothesis does not require the production of such an endogenous psychotogen but seeks to explore the details of abnormal methylations, and the various ways such an abnormality may be associated with or cause schizophrenia.

Recent reviews (15,16) have described the course of the original hypothesis and the many experiments that have evolved from it. The theory has been supported by a succession of experiments which lend credulity to it: the discovery of methylating enzymes by Axelrod (17) and Friedhoff (18); the report of DMPEA by Friedhoff and Van Winkle (19) and the ensuing controversy over the existence of this metabolite in the urine of schizophrenics; the finding by more than ten independent laboratories that feeding of methionine to schizophrenics worsens their psychosis (20); and our finding of abnormal oxidation of the methyl carbon of methionine in patients with schizophrenia in vivo (21), and in cultured leukocytes of such patients in vitro (22).

Further studies by Friedhoff and his co-workers (19) have indicated that there are two forms of catechol-O-methyltransferase (COMT), the enzyme that normally catabolizes dopamine by methylating it, and that one form of the enzyme might be capable of forming DMPEA or other, possibly psychotomimetic compounds. We have reported on the demethylation rates of DMPEA and the trimethoxylated psychotomimetic TMPPIA by labelling with ¹⁴C and measuring expired ¹⁴CO₂ in rats (25,26). Addition of a third methoxyl group greatly increases the amount of demethylation, and in recent work now ready for publication we found that demethylation at the para-position appears to be greatly diminished in brain compared to the periphery. This would seem to make the brain unusually sensitive to para-methoxylated compounds, being unable to inactivate them. It was our earlier observation of the importance of the para-position (23) which led us to the synthesis of the iodoamphetamines and the discovery of their striking uptake in brain.

Our previous work noted above (21,22) and the clinical worsening caused by methionine feeding indicate an abnormality of methyl metabolism in schizophrenia not dependent on the postulate of an endogenous psychotogen. Several other hypotheses have been put forward that also do not require such a postulate. Barker et al. (28)

have suggested what they term the hyperformaldehydism theory, which postulates an abnormality not further defined, in the oxidation of methyl groups at the level of the "active formaldehyde" components of the one-carbon cycle. Levi and Waxman (29)

have proposed that schizophrenia is caused by a deficiency of adenosylmethyltransferase, the enzyme that provides the sole source of the body's methyl groups by connecting methionine to S-adenosyl methionine (SAM) (Fig. 2, reaction 1). Among the many functions of SAM is to provide methyl groups for inactivation of dopamine by COMT. A reduced ability of the brain to perform this function could lead to a hyperdopaminergic state, especially in times of crisis with increased demand for dopaminergic activity, as called for by the dopamine theory of schizophrenia. Levi and Waxman also note that methyl groups are required in greater than normal amounts by malignant cells, and that a number of workers have reported markedly lower rates of cancer in populations of schizophrenics, which they propose would be accounted for by the deficiency of methionine adenosyltransferase. One group of workers has reported that the activity of this enzyme is lower than normal in erythrocytes of schizophrenics (30). Probably the best summary of the current knowledge of the labile methyl

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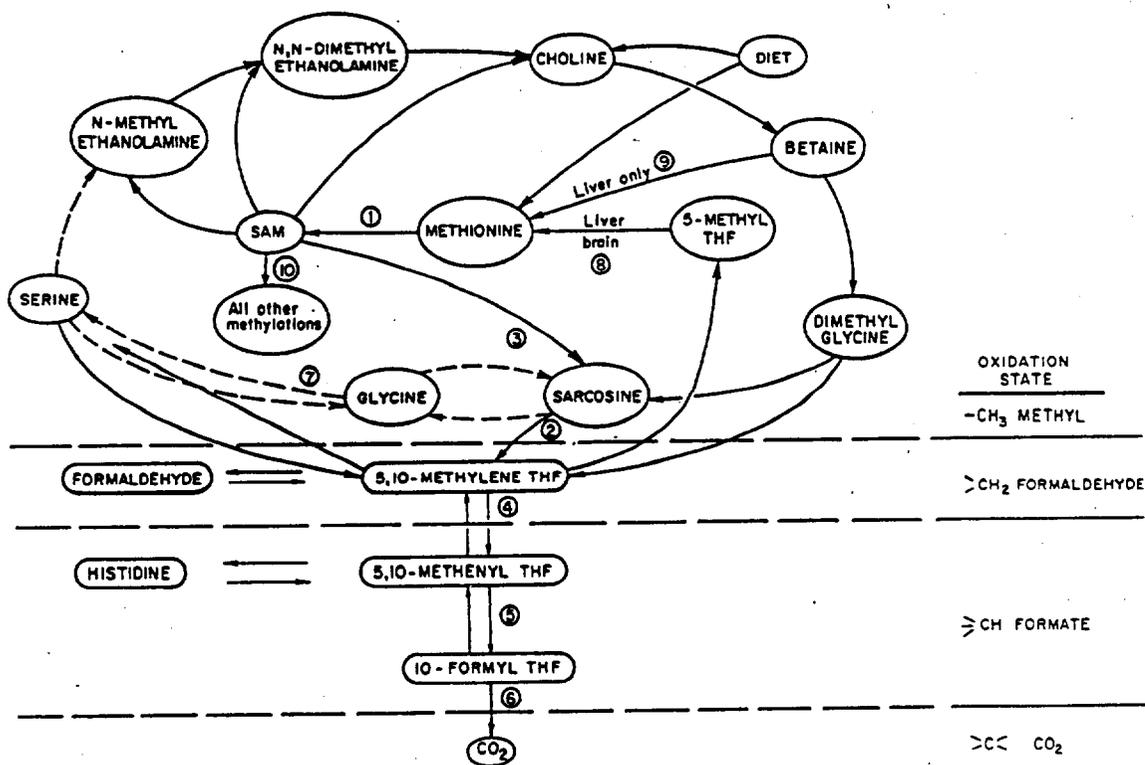
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20. DETAIL ATTACHMENTS:

SUMMARY OF ONE-CARBON OXIDATION STATES



XBL821-3554

FIG. 2. This summary of one-carbon oxidation states is intended to show principally the pathways of the methyl group, which has the oxidation state -CH₃, and more briefly the progression to CO₂. Not all intermediates and side pathways are shown.

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20. DETAIL ATTACHMENTS:

Attachment d. Background (continued):

pool is in two recent papers by Mudd and co-workers (31,32). Our own version of methyl carbon pathways is shown in Fig. 1, in a manner which separates the one-carbon oxidation states, namely the methyl, formaldehyde, and formate states. A labile methyl group is any methyl moiety which can be transferred from one molecule to another, without changing its oxidation state, by normal metabolic processes. This includes the methyl of S-adenosylmethionine, of N⁵-methyltetrahydrofolate (N⁵MTHF) and one of the methyl groups of choline and betaine (Fig. 2). De novo synthesis of methyl groups also occurs, principally from the alpha carbon of serine and the imidazole carbon of histidine. These carbons must be reduced, and enter the methyl pathway only via 5-methyl THF. Mudd has calculated minimum values for net methyl utilization in various pathways, based on dietary balances studies (31,32) and, in part, on our measurements of methionine methyl oxidation (21). When dietary methionine intake exceeds the amount necessary to satisfy the basal levels, the excess methyls are used for methylating glycine to form sarcosine (Fig. 2, reactions 2 and 3), which in the sarcosinemia patient is excreted in the urine; in normal subjects it is oxidized eventually to CO₂ via "active formaldehyde" and formate (Fig. 2, reactions 4,5, and 6). By measuring the amount of sarcosine excreted by these patients under various levels of methionine in their diets, he was able to determine the amount of methionine methyl groups that normally pass through this route but are not detectable because of the normally rapid oxidation of sarcosine.

Studies on methionine metabolism in animals (33) had shown that when methionine is fed in increasing amounts in the diet, oxidation of the S-methyl carbon to CO₂ is increased. We have confirmed this in animals and in human subjects (see attachment f.). This is in agreement with the conclusion of Mudd that removal of methyl groups that are not required for methylation reactions is via sarcosine, serving as the overflow outlet for excess methyl groups. The ¹⁴CO₂ that we observed from methionine which displayed abnormal kinetics in schizophrenics (21) presumably passed via sarcosine, and for this reason ¹⁴C-methyl labelled sarcosine may become of interest in our studies. We have studied the oxidation of ¹⁴C-methyl sarcosine, and of other labelled steps in the route to CO₂, in rats (see attachment f.), and found that the limiting step in the pathway appears to be in transfer of the methyl group from methionine to sarcosine.

There are a number of reports of differences in metabolism of methyl groups in brain compared to liver: These include serine hydroxymethyltransferase (Fig. 2, reaction 7), also found deficient in schizophrenic's red cells (30); methionine methyltransferase after methionine loading (Fig. 2, reaction 1)(36). Methylation by SAM yields homocysteine which is remethylated by 5-methyl THF in both liver and brain (Fig. 2, reaction) but by betaine (Fig. 2, reaction 9) only in liver.

Because of these differences in brain and liver enzymes, we plan to measure the kinetics of ¹⁴C-methionine in brain and liver by whole-body scanning. A difference in brain compared to liver may be seen between normals and schizophrenics, which will appear as differences in the kinetics of methionine metabolism in the two organs.

The supply of methyl carbons seems to be regulated by complex feedback control among several enzyme systems, with the principal exits from the pool being via SAM to a multitude of methylated products (Fig. 1, reaction 10), while sarcosine appears to operate as the overflow when a surplus occurs. There are other possible fates of oxidation of the methionine methyl group, such as the transamination pathway leading to 3-methylthio-propionic acid. The extent to which this and other pathways are involved in normal in vivo metabolism is still unknown, but any such known or unknown

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Attachment d. Background (continued):

pathway could be involved in the abnormal methionine methyl oxidation seen in schizophrenia. It is the purpose of this proposal to seek to identify such pathways if they exist, or to determine how a disturbance of known pathways could be related to or be the cause of schizophrenia.

Attachment e. Approach

1. Regional cerebral blood flow. There is a widespread need in both clinical and research studies for a simple, rapid and accurate measure of rCBF, as outlined in d. We propose to develop a method for synthesis of a radiopharmaceutical for making such a measurement with PET, and to compare the measure of rCBF as determined by this agent with measures of rCMR using ^{18}F FDG in the same patients. We propose to verify the generally held view that, except in specific disease states, rCBF and rCMR are coupled and that the much simpler and sequentially repeatable measure of rCBF can be used to assess altered brain metabolism.

The radiopharmaceutical that we propose to develop will be a congener of the 4-iodo-2,5-dimethoxyamphetamines that we developed earlier (4,5,6). We have investigated two such compounds as described in attachment f, and we will investigate a number more to choose the one with the optimal total brain uptake and brain/blood ratio. The studies by Winchell et al. (7,8), although they examined a wide variety of iodine labelling positions and other variations in the molecule, reported a rather limited number of variations on the para-iodo amphetamine type of structure, of which the secondary amines principally the N-isopropyl amine, had the highest brain/blood ratio (approximately 20:1). While the brain/blood ratio is an important parameter, the blood content of brain is only about 6%, so an improvement in the ratio from 10 to 20 changes the amount of isotope remaining in the brain blood pool from 0.6 to 0.3%, both small in terms of imaging error. As reported in attachment f., our newest compound, 4-iodo-2,5-dimethoxy-N,N-dimethyl amphetamine (IDNNA) has a ratio of 9:1, and while we will seek other congeners to improve this ratio, this molecule has significant other advantages that we will maintain and utilize. These advantages arise from the fact that the 2,5-dimethoxy ring substitution in the uniodinated precursor provides a sterically imposed direct iodination at the para position, so that the labelling reaction with ICl is a) very rapid, less than 1 min, and b) carrier-free.

The compounds reported by Kung and Blau are labelled by exchange, and although the synthesis used by Winchell et al. has not been reported it is probably also labelled by exchange. The rapid and carrier free labelling of our compounds can occur when the nitrogen is di-substituted and hence the ICl does not attack the nitrogen, and the final product is produced in a single rapid step. These characteristics provide a potential not otherwise possible labelling at very high specific activity with very short half life carrier-free ^{122}I , a positron emitter with $T_{1/2}=3.6$ min, which is the daughter of ^{122}Xe , a suitable generator isotope with a half-life of 20 hrs.

A generator suitable for labelling our compound was developed several years ago by Richards and Ku at Brookhaven (39). The series of xenon isotopes 121, 122, 123, 125, and 127 are produced in a 2 hr bombardment of ^{127}I by protons, and are transferred to a holding chamber where all of the iodine daughters, including the desired ^{122}I , build up in the chamber.

When required for use, the Xe is cryogenically pumped to a second (reaction) chamber containing chlorine, and as the ^{122}I builds up it reacts with the chlorine to

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Attachment e. Approach (continued):

form ICI and deposits on the walls. When sufficient ^{122}I has built up, the Xe is pumped back to the holding chamber, leaving the ^{122}ICI and a very small amount of the undesirable long half life iodine isotopes in the reaction chamber. We propose at this point to add our precursor compound with the appropriate solvent to the chamber; in one minute or less the reaction is complete, as we have shown in our work on the synthesis of IDNNAs and the reaction mixture will be passed through a column for purification and a millipore filter and be ready for use. Development of the reaction conditions, purification methods, and the optimal N-substitution patterns will constitute a significant portion of our efforts in FY 82.

The IDNNA class of compounds has a number of significant virtues in terms of clinical and research medicine: a) The short half life provides the possibility of measuring rCBF within time periods of a few minutes, and repeat studies at intervals of 15 or 20 minutes. With present ^{18}FDG studies, it is necessary to wait at least 30 minutes after injection for the blood level to decline before measuring the rCMR. Since these studies have demonstrated changes in rCMR with sensory stimulation, it is clearly difficult to maintain constant stimulation and cognitive status in patients with dementia or schizophrenia for such periods of time. IDNNA will make possible rCBF measurements to compare with ^{18}FDG rCMR, requiring only a few minutes of constant mental status; repeat studies after 15-20 minutes under changed status can then be done allowing direct comparison of such changes in the same patient at close time intervals. Subjects will be studied in mental states established by specific mental task training developed by our colleagues at the Palo Alto Veterans Administration Medical Center (PAVAMC). The low patient dose due to the short half life will be a further advantage.

b) For use of the rCBF determinations in conjunction with our further studies of brain metabolism using ^{11}C compounds it will be important that the data be acquired with the same equipment, namely the PET ring camera, so that the data will be directly comparable. For this a positron emitter is required, and ^{122}I is the only feasible iodine isotope. ^{18}FDG , in addition to the problems noted above, must also be produced by a cyclotron, and production of both ^{18}F and ^{11}C compounds within a short time, although possible with changeable cyclotron targets, would be an additional difficulty. Use of the ^{122}Xe generator eliminates one of these production steps.

c) More widespread use of PET systems is limited by the requirements to have an operating cyclotron near by to produce positron isotopes. The 20 hr half life of the ^{122}Xe generator is sufficient to make possible production at central cyclotrons and shipment to PET facilities. The short half life of ^{122}I would mean reduced patient doses, and many patients could be studied from each preparation of the generator.

d) Because the iodination is direct there is no necessity for carrier or the presence of cold iodine to be exchanged, and the specific activity will be very high; the theoretical specific activity of ^{122}I is 5×10^{13} Ci/mole. Thus, the chemical quantities of IDNNA to be used will be so small that pharmacological problems will be obviated.

We will compare our IDNNA compounds with the IAMPH compounds of Mitchell and the HIPO compound of Kung and Gau in animal studies, using ^{123}I or ^{131}I for scanning camera or organ distribution studies and ^{124}I for PET studies. After organ distribution studies by serial sacrifice in rats, image quantitation will be studied in dogs and monkeys, and correlated with injected microspheres to compare accurately the ability

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Attachment e. Approach (continued):

to measure flow. At the same time the ¹²²I generator system of Richards (39) will be developed and modified for labelling the compound we find to be the best flow indicator. Yukio Yano in our group has had extensive experience and success in development of very short lived isotopes from generator systems, especially the ⁸²Sr-⁸²Rb and ⁶⁸Ge-⁶⁸Ga systems, and will collaborate on this aspect of the program.

The required toxicology and pharmacology for an IND will be done by contract to a commercial laboratory. Approval by the UC Committee for Protection of Human Subjects will be obtained and the granting agency notified before human studies begin. Human studies will be ready to start by the end of FY 82.

The ¹²²IDNNA system will be studied in two collaborative programs already established. These are for study of rCMR by ¹⁸F DG in patients with senile dementia with Dr. Robert Friedland of the Martinez Veterans Administration Medical Center and in patients with schizophrenia with Dr. Phillip Berger and Dr. Adolf Pfefferbaum of the Schizophrenia Biological Research Center at the Palo Alto Veterans' Administration Medical Center (PAVAMC). These studies will attempt to verify or refute the reports of reduced metabolic activity in the frontal region of patients. CT scans will be used in all human studies to enable correlation of rCBF with morphologic images.

The CT/PET positioning device is a head holder fitted with a mask of heat-malleable plastic which is custom-moulded to the face of each patient. It covers the forehead to the bridge of the nose and is equipped with cut-outs for the eyes. Index marks on both mask and patient guarantee accurate repositioning of the mask for staged studies, as well as insuring directly comparable planes of section for the x-ray CT and positron emission tomographs. The device fits into exactly equivalent positioning holes in both the CT scanner and the PET ring.

2. Transmethylation and Catecholamine pathways. This project is designed to utilize at one time, on each patient, a simultaneous series of studies which will exploit the unique advantages of the high specific activity and short half life of carbon-11, synthesized into the methyl group of L-methionine and administered to the patient for a virtually noninvasive, in vivo study of the kinetics of the methyl group. The sequence and timing of the entire experimental sequence described below is clearly complex and difficult. It may be noted, however, that failure of certain parts of the total experiment will only mean a loss of some of the data, and the remaining data will still be useful. The first year of the proposal will be spent in developing each part of the study to high reliability, on animals where appropriate. In FY 84 we expect to complete the full set of studies on each patient. Our prior experience in complex patient studies has demonstrated that they are possible and that significant data not obtainable any other way can be acquired.

The techniques to be employed in this study will include: a) CT scan; b) rCBF measurement with ¹²²IDNNA; c) synthesis of [¹¹C-S-methyl]-L-methionine and injection into patients and normals, who will be studied by d) whole-body scanning for brain/liver kinetics and in later studies by e) positron tomography studies of regional brain kinetics of ¹¹C; f) sequential plasma sampling and HPLC analysis to obtain time/activity curves of metabolites; g) ¹¹CO₂ respirometry; h) data analysis and compartment modelling. When all of the study methods have been developed data acquisition will be time synchronized and stored on the PDP 11/34 which serves the PET system.

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Attachment e. Approach (continued):

Following is a summary of each of the methods to be employed in the program:

a) CT scans will be performed on the EMI 70-70 scanner (resolution 1 mm) adjacent to the PET scanner room, on all subjects who will receive $^{122}\text{IDNNA}$ and/or ^{11}C methionine PET scans. Three planes will be scanned at OM + 4, OM + 5, and OM + 6, from which our radiologist and neurosurgeon will select the optimal plane to include, as nearly as possible, the frontal lobes, caudate nucleus, hippocampus, parietal, and occipital regions, and a fourth scan will be made at the chosen angle and distance above the orbital-meatal line to confirm the choice. The head holder we have designed fits both the EMI scanner and the PET scanner so that the plane of section may be reproduced exactly when the subject is positioned in the PET scanner. The CT scan is essential because our experience has shown that external markers are insufficient to define the location of cerebral structures.

b) Measurement of rCBF with $^{122}\text{IDNNA}$ will be performed as described in detail in section 1 of this attachment. This study will be done as a single study in some subjects during the development phase and quite probably in separate programs which will be designed to measure rCBF under other conditions such as stroke, dementia, and other neurological disorders.

c) Synthesis of [^{11}C -methyl]-L-methionine. The method of synthesis was originally devised by Comar and his co-workers at Orsay (23); ^{11}C is produced in a target containing 99.9% N_2 and 20 ppm O_2 by the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ reaction using 25 Mev protons at the LBL 88" cyclotron. It is reacted with LiAlH_4 to form ^{11}C -methanol, then ^{11}C -methyl iodide which is reacted with homocysteine to form the [^{11}C -methyl]-L-methionine. The methionine so produced is the natural L-isomer because the optical center has not been involved in the synthesis. The synthesis involves manipulation of curie levels of radioactivity, and for operator safety, consistency and simplicity of operation has been automated for operation within a shielded cave (40).

d) Whole-body scanning will be done with the Anger Mark II machine which provides a whole-body image with 1.5 cm resolution, obtainable at scan durations of .75 min and quantitation into an HP 5401 Scintillation Data Analyzer. After injection of approximately 1mCi of ^{11}C -methionine, (the maximum capacity of the machine due to its high sensitivity), sequential scans will be made at 1, 2, 3, 5, 10, 15, 20, 30, 50, 70, 90, 100 and 120 minute intervals with increasing scan times appropriate to decay. The sensitivity of the scanner is sufficient to obtain data with the 15 μCi remaining at 120 min. Blood samples will be drawn at comparable intervals (see f below) and ^{11}C respimetry performed continuously (see g. below). Regions of interest over any desired body region can be used to obtain kinetics of the isotope from the data stored on tape and disc, for use in compartment analysis (see h. below). We will pay particular attention to the kinetics in the liver and brain because these organs have different enzyme complements for methyl metabolism as noted earlier.

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Attachment e. Approach (continued)

e) Positron Tomography to study the regional kinetics of ¹¹C-methionine in brain will be undertaken in FY 84, if the whole body scanning, plasma HPLC and ¹¹CO₂ respirometry indicate an abnormality that may be detectable in brain. The ¹¹C study will immediately follow a ¹²²IODINA rCBF study of the patient, and will include blood sampling, ¹¹CO₂ respirometry and compartmental analysis as described for d) above.

The data in the image are free of effects of activity in overlying tissue, a serious liability in all previous radionuclide imaging methods, in terms of quantitation, and the data are in a memory system from which it can be extracted for regional analysis and manipulated by programs written for any desired computations. The Donner PET system currently uses a fixed ring of 280 bismuth germanate crystals and hard-wired data processing equipment and a specially designed histogrammer. The resolution of 8 mm FWHM at the center of the image surpasses that of most other machines, and will be improved by a new design innovation by the time this project begins, to give a resolution of 6 mm (47). Because the detectors do not move as they do in other machines, there is no lower limit on the time interval for each image, and data accumulation can be gated for data from a moving organ such as the heart. This allows measurement of very rapid kinetic phenomena if sufficient activity is present for adequate statistics.

f) Plasma HPLC analysis. The special virtues of this technique when applied to high-specific activity and high total radioactivity radioisotopes used to label molecules, specifically ¹³N and ¹¹C, were first realized and developed by Prof. Kenneth Krohn and his colleagues at Crocker Nuclear Laboratory on the Davis Campus of the University of California. This technology is being transferred to our laboratory by virtue of Prof. Krohn spending a sabbatical year here, and one of his colleagues, Dr. Chester Mathis, spending 2 years here on a postdoctoral training grant.

The basis for the power of the technique is the use of the very high specific activity possible with ¹¹C, of the order of 500 mCi/micromole, and the large quantities of activity which can be injected into subjects while remaining within an acceptable radiation dose. Krohn and Mathis (43) have found that if as little as one atom in 10⁷ of the administered ¹¹C is found in a particular metabolic product, that product can be detected by HPLC after injection of 10 mCi of activity into an animal. This sensitivity will be somewhat less when the dose is diluted by the larger blood volume of a human subject. Thus, the general method that they have developed (43,44) is potentially applicable to detection, measurement, and characterization of metabolic products of the [¹¹C methyl]-L-methionine that we will be administering to our normal and schizophrenic subjects.

The sensitivity for a particular compound will depend on the pool size and turnover of the compound, as well as the fraction of the methyl pool transferred to that compound in the first 20 min after injection (S-adenosyl methionine has a turnover less than 10 min). The fractional distribution and turnover half times are largely unknown.

The potential sensitivity of the method may be estimated as follows: injection of 15 mCi into a human subject with a dilution volume of 5 x 10⁴ cc gives a concentration of 6.6 x 10⁵ cpm/cc. The minimum detectable activity of a well crystal with the HPLC outlet passing through it is about 30 dpm, and after injection of 0.3 cc of plasma the sensitivity is 100 dpm/cc. Thus, 100 ÷ 6.6 x 10⁵ = 1.5 x 10⁻⁴ or .015%

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Attachment e. Approach (continued)

(assume no decay), so the method should be able to detect metabolites containing .015% of the injected methionine. It may actually be more sensitive than this because the dilution volume may be much smaller, and metabolites with a high rate of turnover will be labeled at a higher specific activity. According to Mudd and Poole (31) average daily intake of methionine is 10 meq, and the average daily excretion of labile methyl moiety as catecholamines and derivatives is .04 meq, or 0.4%. If we are able to detect .015% of the injected methyl moiety, we will be able to detect metabolites which are about 4% of the total catecholamines. As noted, the sensitivity may be greater than this. The sensitivity for other metabolites in the metabolic sequence of methyl groups (Fig. 2) cannot be estimated at present because there is insufficient data available, and it is expected that data on relative transfer rates in these pathways will result from the proposed experiments.

In the operational sequence of the system in an experiment, serial venous blood samples will be drawn at intervals comparable to those of the image sequence (electronically marked as was the injection): Heparinized whole blood samples will be drawn, counted, and centrifuged. The supernatant plasma will be removed from the cells and the two fractions counted. The plasma proteins will be removed by precipitation and counted. The protein-free plasma supernatant will be screened by two or three parallel HPLC systems with different columns (e.g., anion exchange, cation exchange, normal and reversed phase) to identify the labeled compounds. Each system will be designed for optimum separation and identification of the labeled metabolites by appropriate choice of solvents and columns. Three separate and parallel HPLC systems will probably be required to efficiently analyse the plasma samples within the half-life of ^{11}C .

For the experimental run, radioactivity detectors on each HPLC system will record the radioactive peaks; by comparison to the reference standards we will attempt to identify the labeled metabolites and the area under the peaks will provide the relative radioactivity in that sample. There will then be a collection of chromatograms for each plasma sample from which the quantities of each molecular species can be determined. Samples drawn up to 1 hr after ^{11}C -methionine injection will have sufficient activity for analysis.

Our earlier report (22) indicated that the methyl oxidation abnormality also occurs in leukocytes. We propose to apply the HPLC method to study cultured leukocytes of schizophrenics. Calculating as previously for human plasma, if we add 10 mCi of ^{11}C -methionine to a culture containing 0.01 cc of leukocytes (10 cc of whole blood), and inject .001 cc of lysed cells into the HPLC at various time intervals, the sensitivity will allow detection of 10^{-9} of the injected label, and yields its time course of production. Because of the relative simplicity of this method and its potentially great power in metabolism measurements, we will apply it in the project upon development of the first methods for separation of the simplest metabolites.

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Attachment e. Approach (continued)

g) $^{11}\text{CO}_2$ respirometry. $^{11}\text{CO}_2$ and $^{14}\text{CO}_2$ radiorespirometry and its applications in our research have been reported (21,45). We have demonstrated its ability to measure exhaled $^{11}\text{CO}_2$ from ^{11}C labeled compounds over a period of 6 half lives of ^{11}C . The equipment is now positioned in the same room as the PET and whole body scanners and in FY 82 will be used for the first time in simultaneous whole-body scanning respirometry studies. This will provide important additional measured parameters for the compartment solution of the kinetics of brain metabolites..

In FY 83 simultaneous PET-respirometry studies will begin. In preliminary studies with ^{11}C -methionine in a group of schizophrenic patients not well categorized or diagnosed clinically, we have found the same type of $^{11}\text{CO}_2$ respiration patterns that we found with ^{14}C -methionine (21).

h. Data analysis and compartment modelling. As a general rule, all data will be recorded directly into the PDP 11/34, or onto magnetic tape in format compatible with the PDP 11/34. This will include CT scans, PET scans of $^{122}\text{IDNNA}$ and ^{11}C -methionine, and $^{11}\text{CO}_2$ respirometry. The HPLC plasma data output will be digitized and recorded directly into the PDP 11/34, using hardware and software systems developed at UC Davis. The CT scan will be recorded on tape compatible with the PDP 11/34 and with the computer used at the NIH-MHCRC at the Palo Alto VA. In this way brain areas may be outlined on a visual basis or a computer-outlined density contour basis, and the same area used for quantitation of the ^{11}C - or $^{122}\text{IDNNA}$ PET images.

The $^{11}\text{CO}_2$ respirometry data are handled by a program developed at this laboratory (45). Data sampling as described previously is digitized and recorded on magnetic tape and the program subtracts background, corrects for decay, calculates specific activity and multiplies by the mean CO_2 for the entire experiment, thus correcting for changes in respiration rate, yielding curves of percent of injected dose/min as a function of time. The program then calculates by an iterative least squares method the best fit of the desired exponential function, and calculates the parameters of the function, the integral and extrapolated $^{11}\text{CO}_2$ excretion. A method for calculation and correction for the input function of the $^{11}\text{CO}_2$ respirometer, just developed by Dr. Budinger of our group (46), will be incorporated into the program.

The HPLC elution data output will be in the form of three sets of elution curves of radioactivity peaks vs. elution time, for each plasma sample. From the sequence of curve peaks and knowledge of the established metabolic pathways, estimations may be made of pool sizes and transfer rates, and possibly some guidance obtained for identification of unknown peaks.

Relating the HPLC plasma data to the kinetic data will be limited by two factors. One is that the plasma does not directly represent liver or brain. Second, one or two metabolites may be so dominant in the plasma that if these are inferred to be also present in the brain they will mask the metabolism of others in the compartment model. If one of the dominant metabolites is abnormal in the schizophrenic subjects we will have achieved one of our principal goals. Our earlier radiocarbon respirometry data does show abnormal oxidation of the methionine methyl group; thus, even though this may be masked by other metabolism in the brain and whole body images, it is likely that we will be able to detect an abnormality in one of the plasma functions that will correlate with the expected abnormal oxidation.

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20. DETAIL ATTACHMENTS:

Attachment e. Approach (continued)

Information from all three systems (^{11}C , tomographic and whole body images, and plasma HPLC) will be inputs to the computer modelling system. In our previous experience with modelling of data, we have found that data which at first glance appear to measure only a global, large scale phenomenon can provide important information for the solution of a model, by providing what amounts to a bookkeeping balance and significantly reducing error in the final solution. Dr. Ronald Huesman, mathematical physicist in our group, has developed a program which is a generalized model system. Virtually any model within reasonable limits can be built with the system, data entered for the compartments measured, and solutions for transfer constants obtained. Thus, different model systems can be tested with any set of data, and the closeness of fit can be tested for the final parameters. In addition, the SAM program package is also available.

If our results indicate that it is important to measure more variables, or that the metabolic abnormality of schizophrenia is located at another point in metabolism, we have the possibility of synthesizing other metabolites with carbon-11.

These include a method of labeling of amino acids on the carbonyl atom of amino acids developed in our group (48); [^{11}C -N methyl] sarcosine by methylation of glycine with ^{11}C -methyl iodide; and ^{11}C -formaldehyde as developed by Comar's group (49). We have also begun to develop a method for labeling tyrosine in the beta position, so that in the conversion of the tyrosine to the neurotransmitter catecholamines, the label will remain with the core of the molecule. The method could allow labeling with ^{11}C in a one-pot synthesis. Although we are presently interested primarily in methyl metabolism, we hope to be able to develop this radiopharmaceutical for investigation of catecholamines in vivo with PET and HPLC methods.

The approach outlined above constitutes a combination of unique capabilities in use of radioisotopes for in vivo studies of disease in humans, applied in this project to the uniquely human disease of schizophrenia, but applicable in principle to the study of a variety of metabolic dysfunction in man. We feel that its development will contribute not only to an understanding of mental disorders but will serve as a model approach to other problems of the biochemistry of disease.

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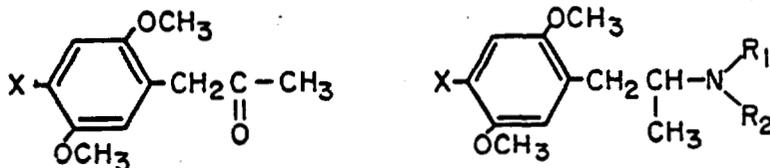
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20. DETAIL ATTACHMENTS:

Attachment f. Technical Progress

Our investigation of the new IDNNA class of iodoamphetamines for measurement of rCBF in FY 82 proceeded along the lines of studying the chemistry by investigating the chemistry of rapid synthesis to develop the very rapid synthesis necessary, and brain and organ distribution in animals to determine whether molecules suitable on the basis of fast synthesis had the requisite brain uptake. The basis upon which we are proceeding is by making the appropriate substitution on the amine group and thereby protecting the nitrogen from attack during iodination while the para position is iodinated. The resulting compound will thus have the desired brain uptake and will not require the removal of the group protecting the nitrogen as was required in our first iodoamphetamines.

1. IDNNA Chemistry. The first compounds studied were the N,N-dimethyl and the N,N-diethyl analogs (e and f, Fig. 3). These compounds were prepared from precursors d and g respectively by direct iodination with $^{131}\text{I}\text{Cl}$ under acidic conditions. The iodine free dimethyl compound, d was prepared by reductive amination of k with dimethylamine; g, the diethyl compound, was made by reductive alkylation of b with acetaldehyde. Both reductions employed NaCNBH_3 .



	X
(k)	H
(l)	I

	X	R ¹	R ²
(b)	H	H	H
(d)	H	CH ₃	CH ₃
(e)	I	CH ₃	CH ₃
(f)	I	C ₂ H ₅	C ₂ H ₅
(g)	H	C ₂ H ₅	C ₂ H ₅
(h)	H	-CH(CH ₃) ₂	H
(i)	H	C(CH ₃) ₃	H
(j)	I	H	H

FIG. 3

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20. DETAIL ATTACHMENTS:

Attachment e. Approach (continued)

TABLE 1. PRELIMINARY ORGAN DISTRIBUTIONS IN RAT

		1 min	5 min	15 min	30 min	
cmpd e	% inj.	blood	0.21	0.20		0.11
		brain	0.58	1.8		0.54
		brain/blood	2.8	9.0		4.9
	dose per gm.	liver	0.28	1.1		0.89
		lungs	3.4	3.1		1.7
		kidney	1.2	2.4		0.92
		heart	1.2	0.84		0.26
		muscle	0.36	0.20		0.15
		thyroid	1.5	4.3		1.8

cmpd f	% inj.	blood	0.09	0.97	0.05	0.07
		brain	0.18	0.39	0.19	0.17
		brain/blood	2.0	5.6	3.8	2.4
	dose per gm.	liver	0.14	0.45	0.38	0.38
		lungs	1.4	2.0	1.4	1.5
		kidney	0.45	0.69	0.38	0.51
		heart	0.36	0.35	0.15	0.16
		muscle	0.19	0.08	0.07	0.07
		thyroid	0.52	0.74	0.82	1.2

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20. DETAIL ATTACHMENTS:

Attachment f. Technical Progress (continued)

We have prepared the precursors for compounds h and i and are studying the iodination reaction of each. We propose to study a series of N-substituted analogs with the following strategy. The secondary amines will be made by preparing compound l with ^{131}I , and then reacting aliquots of it with the appropriate primary amine to generate N-substituted analogs. Correspondingly, synthesis of ^{131}I labeled j will allow reaction with a variety of aliphatic aldehydes to produce a series of N-disubstituted analogs. Each labeled analog so produced will then be tested for brain, blood and organ uptake as previously done for e and f. Those with the most promising brain/blood levels and total uptake will then be evaluated more extensively by imaging studies in dogs.

2. IDNNA-Labeled studies in animals. Organ distribution studies of compounds e and f labeled with I-131 were performed in male Sprague-Dawley rats weighing 300-400 g. Ether anesthetized rats were injected in a tail vein with 10-20 μCi of the I-131 labeled compound. Several blood samples were drawn by cardiac puncture and the rats killed at selected intervals by decapitation. Each organ to be assayed was weighed wet and counted in a $\text{NaI}(\text{Tl})$ scintillation counter. The organ distribution data from at least two rats were averaged to yield the data in Table 1.

The % administered dose per gram of brain tissue and the brain to whole blood activity ratios were greatest at 5 minutes for both I-131 compounds. These values decreased by approximately 50% at 30 minutes indicating slow loss of tracer from the brain. The N, N-dimethylamphetamine exhibited higher absolute brain uptake values and higher brain-to-blood ratios than the N,N-diethylamphetamine despite the greater lipophilicity of the diethyl compound, as measured in butanol/water partition experiments. The reasons for this finding are not clear, but could involve steric hindrance at non-specific amine receptor sites or impaired diffusion of the free diethyl amine across the blood-brain-barrier.

We have utilized compound e labelled with ^{131}I to study brain uptake and plasma clearance in the dog. Brain uptake and blood clearance data from a beagle dog are shown in Fig. 4. Quantitative image data were acquired using the digitized Anger Mark II Whole-body Scanner after injection of 75 μCi of ^{131}I compound e. Data from regions of interest over the brain were taken from five scans over 30 min and blood samples were obtained at more frequent intervals. The brain/blood ratio was 7.5 at 5 min and reached a maximum of 8.7 at 8 min.

Because the chemical synthesis and purification are rapid, and maximum brain uptake occurs within five minutes of i.v. injection when blood activity is low, compound e appears appropriate for labeling with 3.6 min ^{122}I . This positron-emitting radionuclide is the daughter of 20 hr ^{122}Xe , and is thus suitable as a generator-produced isotope which can be shipped to institutions that do not have a cyclotron.

Based on these preliminary results we feel that there is a high probability of developing these or analogous compounds for use as measures of rCBF for research applications and for clinical use as described under attachment e and this program will be vigorously pursued in FY83 and 84.

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20. DETAIL ATTACHMENTS:

Attachment f. Technical Progress (continued)

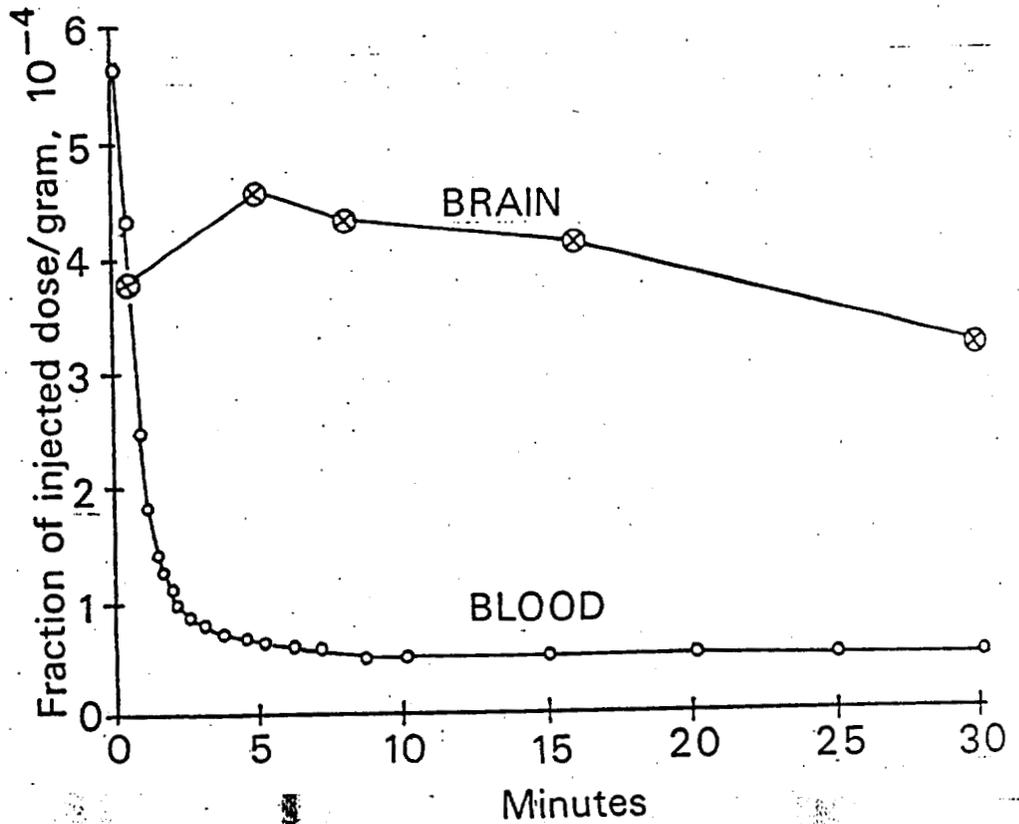


Fig. 4. Brain and blood radioactivity in dog as a function of time after i.v. administration of compound e. Brain activity was obtained from a region of interest over the brain, expressed as a fraction of injected dose per gram of brain. At the 5 min maximum, 3.6% of the total body activity was in the brain.

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20. DETAIL ATTACHMENTS:
Attachment f. Technical Progress (continued)

3. Transmethylation studies in FY 82 have been pursued with [¹⁴C-methyl]-L-methionine while methodologies for HPLC, synthesis, scanning, and collaboration with PAVAMC were being developed and coordinated. An HPLC system with programmable gradient system was acquired and separation methods for metabolites which are expected to be labelled with ¹¹C-methyl groups are being developed.

[¹⁴C-methyl]-L-methionine studies were completed in 3 human volunteer subjects. Each subject was administered the labelled methionine while on his normal diet and the ¹⁴CO₂ excretion measured, and then studied again after four days on either a high or low methionine diet. The diets were designed to deliver approximately normal caloric and protein intake, but with total methionine intake reduced to 1/3 of normal for the low diet and increased to three times normal in the high methionine diet. Results from two of these studies are shown in Fig. 5. The results confirm those from equivalent rat studies reported last year that high methionine diet increases oxidation of the methyl group and the low methionine diet decreases it. The slopes of the exponential components of the curves are changed in addition to the total amount oxidized indicating some change in the oxidation rate as well.

Whether these changes are consistent and statistically significant will have to await completion of a larger number of subjects. However, the results do appear to show that the ¹¹CO₂ and ¹⁴CO₂ curves we observed in schizophrenics given labelled methionine are not likely to have been caused by diet, because the striking changes seen in T₁ in schizophrenia are not produced in human subjects consuming diets as extreme in methionine content as could be readily tolerated.

The principal pathway of methyl oxidation from Fig. 2 was studied in detail in a series of studies on rats. Four rats were each given, in a series of separate experiments, the five compounds shown in the metabolic sequence at the bottom of Fig. 5, labelled with ¹⁴C in the positions shown. This series of metabolic steps is the route taken by the methyl group when it is oxidized to CO₂ as constructed by other workers and outlined in attachment d. Our experiments were extended over a period of 6 hours in order to measure exponential components which do not appear until late in the study. The results for each compound agreed very closely in all four animals, and because the same animal was injected with all five compounds inter-animal variability was greatly reduced. The number of exponential components was found to be four for bicarbonate, three for formaldehyde and formate and two for sarcosine and methionine.

Viewing this pathway in reverse, from bicarbonate to sarcosine, the % of the total injected ¹⁴C expired as CO₂ is somewhat less for each labelled compound in the sequence, indicating some diversion of the label to other points of metabolism en route. However, the yield from methionine is markedly less, supporting the concept of Mudd that the control of oxidation of excess methionine occurs at the first step, the oxidation of methionine to sarcosine. Experiments with cold loading doses of the different metabolites, and HPLC analysis of metabolites in plasma will be used to acquire data to develop a compartment model that will account for the sequence of exponentials and total expired ¹⁴CO₂.

The metabolic pathways of methyl metabolism and their control mechanisms are not well established at present, and a better understanding of them will contribute importantly in many other fields of medical research as well. As noted under background, a hypothesis has been proposed which links schizophrenia to cancer, on the

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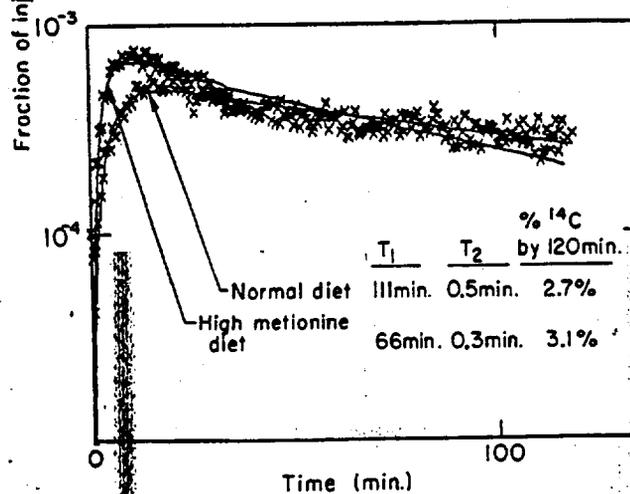
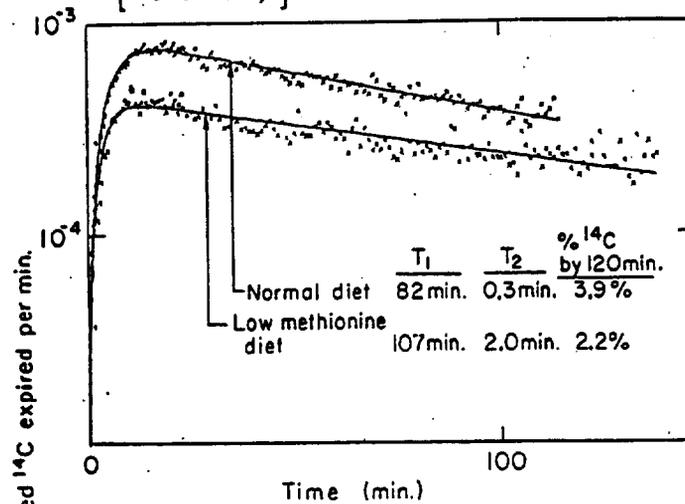
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20. DETAIL ATTACHMENTS:

EXPIRED ^{14}C FROM HUMAN SUBJECTS ADMINISTERED
[^{14}C -S methyl] - L-METHIONINE i.v.



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FIG. 5

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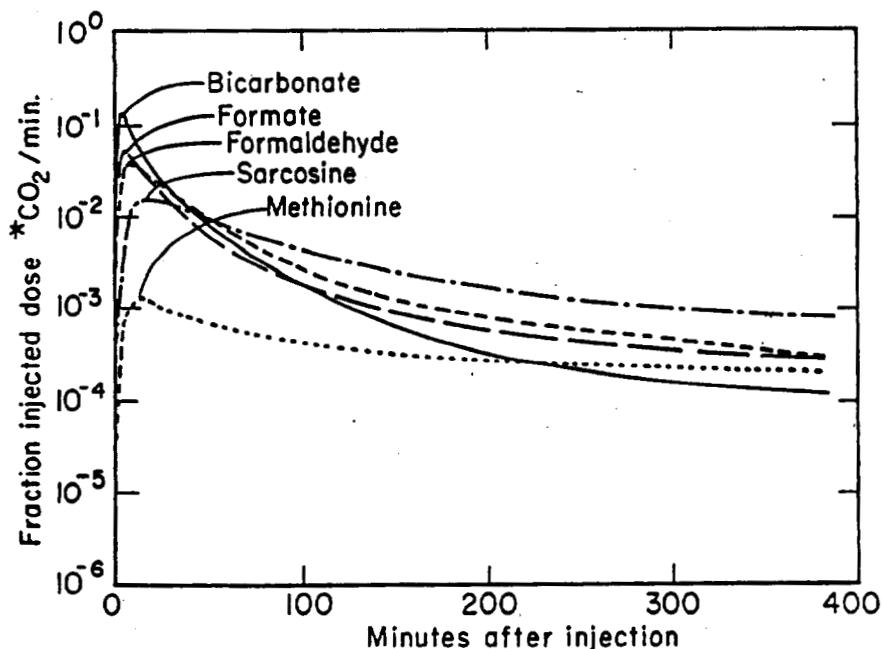
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20. DETAIL ATTACHMENTS:

EXPIRED *CO₂ AFTER INJECTION OF *C-LABELLED
SEQUENTIAL METABOLITES



Position of label injected	$\text{HC}-\text{CH}_2\text{CH}_2\text{S}^*\text{CH}_3 \rightarrow \text{NH}^*\text{CH} \rightarrow \text{H}^*\text{C}=\text{O} \rightarrow \text{H}^*\text{C}=\text{O} \rightarrow \text{H}^*\text{CO}_2 \rightarrow ^*\text{CO}_2$				
	$\begin{array}{c} \text{NH}_2 \\ \\ \text{HC}-\text{CH}_2\text{CH}_2\text{S}^*\text{CH}_3 \\ \\ \text{COOH} \end{array}$ Methionine	$\begin{array}{c} \text{CH} \\ \\ \text{NH}^*\text{CH} \\ \\ \text{COOH} \end{array}$ Sarcosine	$\text{H}^*\text{C}=\text{O}$ Formaldehyde	$\begin{array}{c} \text{OH} \\ \\ \text{H}^*\text{C}=\text{O} \end{array}$ Formate	H^*CO_2 Bicarbonate
2 hr	3.2	33	50	60	92
12 hr	7.5	53	58	67	97
Total % of injected dose excreted as *CO ₂					

XBL821-3552

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FIG. 6

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20. DETAIL ATTACHMENTS:

Attachment f. Technical Progress:(continued)

basis of methylation reactions which are required in excess by malignant cells for their multiplication. This is a large field of research in itself, and work in this project may impinge upon it in future years.

Attachment g. Future Accomplishments:

In FY 83 we will have the ¹²²IDNNA system operational using the optimal chemical form of the radiopharmaceutical as determined in experiments during FY 82 as described in attachment e. We will begin studies to compare rCBF, as measured with ¹²²IDNNA, to rCMF as measured by ¹⁸FDG. The ¹⁸FDG studies have already begun in FY 82 in patients with Alzheimer's dementia in our collaborative studies with Dr. Friedland at the Martinez VA and will begin with schizophrenic patients from PAVAMC in late FY 82. We expect to be able to answer the question definitively during FY 83 as to whether there are specific regional differences in energy metabolism and blood flow in schizophrenia.

We will begin studies with ¹¹C-methionine in patients and normals, using the whole-body scanner. Dr. Huesman, in project 4454/000331 is developing a system by which the PET ring can be used as a whole-body tomographic scanner, and as soon as this is operational it will be used instead of the existing Anger Mark II Scanner. ¹¹CO₂ respirometry studies will be done simultaneously; the appropriate extensions to connect the breath collection helmet from the scanner bed to the respirometer are in place. HPLC methods for separation of at least some of the metabolites we wish to study will also be developed at this time and blood sampling will be done in conjunction with each patient study.

During FY 83 we will continue to perfect our HPLC plasma separation techniques to be able to identify and quantify a greater number of methyl metabolites. Investigation of metabolites by administering ¹¹C methionine to leukocyte cultures will then be initiated because of the grant sensitivity of the method, and the possibility of measuring intracellular metabolism of cells from schizophrenic patients. Methods for ¹¹C synthesis of other metabolites in the methyl chain will be studied for future tracers to be applied in PET studies for more detailed investigation of methyl metabolism. The method we have devised to synthesize [¹¹C-beta] tyrosine will be pursued to provide a labelled catecholamine for brain PET studies.

In FY 84 we expect to have established a picture of methionine kinetics in normals and schizophrenic in terms of whole-body metabolism, and will begin complete coordinated studies with the PET system. If the 4-ring, 7 plane system being designed and built by Dr. Derenzo, in project 4450/002420 is operational, it will be employed to provide simultaneous brain and liver tomographs, as the rings will be separable. The coordinated studies will consist of: a CT scan to provide the correct alignment for the desired image plane; a ¹²²IDNNA study to provide quantitation regional rCBF; injection of ¹¹C-methionine followed by simultaneous measures of regional brain ¹¹C distribution; ¹¹CO₂ respirometry and blood sampling for plasma HPLC analysis. All of this data will be incorporated in a compartment model analysis to provide an overall picture of methyl metabolism and its differences, which we expect to find in patients with schizophrenia. We will be investigating intracellular methyl metabolism by ¹¹C methionine labelling of leukocytes with HPLC analysis. ¹¹C labelling methods for other metabolites, such as sarcosine and tyrosine, will be in

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20. DETAIL ATTACHMENTS:

Attachment g. Future Accomplishments: (continued)

progress and these labelled molecules will be staged for human studies.

Attachment h. Relationships to Other Projects:

This project is closely related and dependent upon other projects in the Research Medicine Group. Synthesis of ¹¹C-methionine and development of the ¹²²IDNNA system is being done in collaboration with Yukio Yano's Experimental Medicine Development Group (4452-000330). The PET system, development of the whole-body scan tomograph system, and compartment modelling is being done principally by Drs. Budinger, Huesman and Derenzo in the Experimental Medicine Clinical group. 4454/000331.

Development of the 4 ring 7 plane PET system which will eventually be used in this program is being done by Dr. Derenzo's Positron 3/D Imaging Instrument program, 4450/002420.

Attachment i. Environmental Assessment: N/A

Attachment j. Explanation of Milestones: N/A

Attachment k. ZBB Detail: N/A

Attachment l. Equipment FY 83:

Gamma counter for radioactive peak measurement from HPLC output. Special fabrication	\$9,000.
High Performance Liquid Chromatography system to provide second parallel track of analysis.	\$9,000.
High speed air driven microcentrifuge, for rapid separation of blood samples.	\$1,200.

FY 84

High Performance Liquid Chromatography System. Required for third parallel track to provide rapid separation of labelled metabolites in different solvent separation systems.	\$10,000
Gamma counter for HPLC output.	\$9,000

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20. DETAIL ATTACHMENTS:

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