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The Medical Research Center
Brookhaven National Laboratory
Upton, L. I., New York

REPOSITORY Records Holding Area, Bldg. 494
COLLECTION Committee-Clinical Investigations and Uses of
Radioisotope
BOX No. 4
FOLDER CIRC# 17

The study we plan to carry on here and for which you have volunteered to assist us is concerned with the testing of new antimalarial compounds which are being developed by competent investigators under a program sponsored by the United States Government (United States Army). The purpose of the testing program is to ascertain whether the drugs are effective and safe for administration to human beings. The nature of the drugs we propose to use is such that we do not expect serious complications from their use. The toxicity of the drugs is unknown or has been incompletely studied as far as human beings are concerned, but no drugs will be used at a dosage which, on the basis of previous tests in animals or men, would lead us to anticipate severe enough toxicity to endanger life or to produce permanent disability. Persons volunteering to assist in this study will be given medication, will submit to frequent blood tests and other laboratory tests, and will be asked to cooperate with us for a period of at least thirty days or for several months, if indicated. Volunteers will have blood drawn at intervals and may also receive transfusions of blood either from normal individuals or from subjects who are susceptible to anemia when treated with primaquine and/or other antimalarial agents. The persistence of red cells in the blood and the presence of other substances will be determined by radioactive isotope techniques which have been approved as safe by the University of Chicago Clinic (Radioisotope Committee).

A fee of Twenty Dollars (\$20.00) will be paid to each individual who is accepted for and who completes the prescribed tests. This fee will be payable within thirty (30) days after tests are started.

ARMY MEDICAL RESEARCH 2413

BY: _____

I, _____ No. _____ being _____

years of age, having volunteered to subject myself to certain scientific studies which have for their purpose the determination of the toxicity and efficacy of new anti-malarial compounds (the nature and extent of which studies, as described in the foregoing statement is fully understood by me) to be conducted by the University of Chicago under a program sponsored by the United States Army, in consideration of the cash payment to be made to me in connection therewith, do hereby assume all risks involved in my participation therein, and acting for myself, my heirs, personal representatives and assigns, do hereby release and forever discharge the University of Chicago, a corporation, its Board of Trustees, individually and collectively, its officers, agents, employees and instructors, and specifically Dr. Paul E. Carson, Dr. Karl Rieckmann, Dr. Henri Frischer, Dr. James E. Bowman, Dr. Lawrence Kass, Dr. William D. Willerson, Dr. Robin D. Powell, all doctors, nurses and individuals who may have any responsibility connected with the University or the United States Army, the State of Illinois, the Director of the Department of Public Safety of the State of Illinois, the Warden of the Illinois State Penitentiary at Joliet-Stateville, and all employees connected with the above institutions and departments who are conducting the studies referred to in the foregoing statement, of and from all suits, claims or demands of every character arising from or relating in any way to such experiments and tests, including personal injury, damage to or loss of health, life or property which I, my heirs, executors, administrators or assign, hereafter may or shall have by reason of my participation in such studies.

I hereby agree to cooperate with any and all doctors, nurses and medical personnel who may be assigned to treat me or attend me, and to take such medications and submit to such treatment as said doctors may prescribe, promptly in accordance with their direction.

This is to certify that this release is being executed voluntarily and under no duress.

In Witness Whereof, I have hereunto set my hand and seal this _____ DAY of _____ 19 _____

(SEAL) _____
Volunteer's Signature

WITNESS: _____ ADDRESS STATEVILLE

WITNESS: _____ ADDRESS STATEVILLE

The Medical Research Center

Brookhaven National Laboratory

Upton, L. I., New York

1179806



UNIVERSITY HOSPITALS

STUDENT INFIRMARY
MARY CORNELIA BRADLEY MEMORIAL
STATE OF WISCONSIN GENERAL
WISCONSIN ORTHOPEDIC FOR CHILDREN
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NEUROLOGICAL AND REHABILITATION

UNIVERSITY OF WISCONSIN MEDICAL CENTER

1300 UNIVERSITY AVENUE • MADISON, WISCONSIN 53706

CONSENT TO TRYPTOPHAN AND/OR KYNURENINE METABOLISM TEST

I, _____, hereby give my consent to Professor R. R. Brown and/or his associates, to administer tryptophan and/or kynurenine by mouth to me for the purpose of studying the ability of my body to utilize these substances. Tryptophan is an essential dietary component of protein required by man, and kynurenine is one of its chief breakdown products which also occurs naturally in the body.

Signed: _____

Date: _____

Witnessed: _____

1179807

The Committee on Clinical Investigations and Uses of Radioisotopes
hereby approves the program with the following title:

Metabolism of Tryptophan

CIRC # 11 has been assigned to this program.

Walton W. Shreeve

Walton W. Shreeve, M.D., Ph.D., Chairman

E. P. Cronkite

Eugene P. Cronkite, M.D.

E. Schackow

Eckart Schackow, M.D.

M. H. Van Woert

Melvin H. Van Woert, M.D.

J. S. Robertson

James S. Robertson, M.D., Ph.D. (ex officio)

Date November 8, 1963

Place Medical Research Center
Brookhaven National Laboratory
Upton, New York

1179808

UW 2713/63

BROOKHAVEN NATIONAL LABORATORY
MEMORANDUM

DATE: Aug 29, 63

TO: Dr. V. P. Bond

FROM: J. J. Van Slyke

SUBJECT: Proposal of L. V. Hanke,

J. W. Joseph, and R. R. Brown for a study of
tryptophan metabolism with C-14 labeled tryptophan
in certain types of patients.

The investigations proposed are well planned
and may add significantly to knowledge concerning
human metabolism, both normal and in the conditions
of the patients. Hanke's experience with micro
methods, and in the handling of tryptophan metabolic
products (e.g. in experiments with mice), makes it possible
to carry out the work with such small amounts
of C-14 that any danger to patients from this
source appears to be excluded. The proposal
seems on all counts to merit approval.

Donald J. Van Slyke

Rec'd 9/10/63

18

V.P. Bowd

FORM FOR INITIATION OR REVIEW OF CLINICAL
INVESTIGATIVE PROGRAMS

(Submit original only to Department Chairman)

- A. Title of the proposal: Metabolism of Tryptophan
- B. Sponsoring physician(s): V. P. Bowd and E.F. Cronkite
- C. Responsible investigator(s): L. V. Hawkes, Ph.D. J. E. Joseph, R.R. Brown, Ph.D. (Wisconsin),
- D. Brief description of the study, including its general goals and purpose, and pertinent information on past studies: (Attach additional sheets if necessary.)
D and E are answered in the attached sheets

E. Reasons why the investigation(s) are to be performed on human subjects.

F. Type of patient in which the study is to be done (including approximate number of subjects, if known; special restrictions or requirements; method of obtaining consent; etc.):

Multiple myeloma-approximate ten patients
Scleroderma-approximate ten patients
Anemia-approximately ten patients

1179810

- G. 1. Are drugs not in the U. S. Pharmacopoeia (USP) or the NNR being used or contemplated for use? Yes ___ No X
2. Is an unusual use of a drug(s) accepted by the USP or NNR contemplated? (An example would be the use of an accepted drug in dosages far exceeding the recommended limits or for purposes distinctly different from the usual indications cited.) Yes ___ No X
3. Are any biological products to be administered that do not bear on their containers or labels notation of approval by the Biological Control Division of the National Institutes of Health? Yes ___ No X
4. Is external or internal radiation other than accepted diagnostic or therapeutic procedures to be administered? Yes ___ No X
5. Are any (other) unusual procedures being performed or proposed which in your judgment may entail a special hazard - particularly a hazard above and beyond any imposed by accepted diagnostic and therapeutic measures for that patient? Yes ___ No X
6. Are any radioisotopes to be administered to human beings? Yes X No ___
- a. If yes, are the radioisotopes to be used solely within the limits of procedures, specifically described in the USP? Yes ___ No ___ ✓
Describe the radioisotopic preparation(s):
- b. Or are the radioisotopes to be used only in accordance with a project previously approved by the former Radioisotope Committee of this Department? Yes X No ___
Note the project number: H-69 and H-69 (Modified)

IF ANY OF QUESTIONS 1 THROUGH 5 ARE ANSWERED AFFIRMATIVELY, a detailed analysis of the potential hazards must be appended, including pertinent bibliographic citations and other relevant information.

IF QUESTION 6 IS ANSWERED AFFIRMATIVELY, a completed supplementary form for Radioisotope Administration to Human Beings must be appended. However, this form need not be filed provided that question 6a or 6b is also answered affirmatively. A separate form must be submitted for each radioisotopic species to be administered.

V. P. Bond
Sponsoring Physician
M. L. ...

Committee on Clinical Investigations and Uses of Radioisotopes
Approval recommended ✓ Date 11/8/63
Disapproval _____ Date _____

V. P. Bond JUN 29 1964
V. P. Bond, M. D.
Chairman, Medical Department

1179811

6/25/63

Tryptophan, a widely distributed naturally occurring essential amino acid, has been the subject of a great deal of biochemical investigation. It is converted to many other compounds of biological significance, some of which no longer retain the indole nucleus. This amino acid is now recognized as the precursor of such diverse compounds as quinolinic acid, nicotinic acid, xanthomatin, xanthurenic acid, serotonin, Col, and many others. Many points concerning the biosynthesis of these biologically important compounds are still obscure, i.e. Tryptophan balance data on growing animals and the distinct requirement for tryptophan for maintenance in the adult, indicates that a fair amount of carbon from this amino acid is disposed of through the lung and kidney each day. Only a small part of the injected indole nucleus is accounted for in the various excretion products of normal mammals. Tryptophan, like other essential amino acids, must be disposed of by a major route leading to aliphatic compounds and finally to CO₂. There is evidence that tryptophan is converted to niacin in man. Niacin deficiency in man has been specifically cured by niacin or tryptophan (3-5) and blood pyridinenucleotides were restored to normal levels by supplements of tryptophan after depletion on niacin-deficient diets (6). A number of intermediary metabolites of tryptophan have been isolated or identified in human urines. Makino and coworkers reported that 3-hydroxykynurenine was responsible for the positive diazo reaction frequently observed in severe tuberculosis (7). Musajo, Benassi and Parpajola (8) isolated kynurenine and 3-hydroxykynurenine from urine of patients with various pathological conditions but could not isolate these compounds from normal human urine. Tuberculosis patients studied by Musajo, Spada and Coppini (9) excreted 3-hydroxyanthranilic acid in significant amounts whereas normal urine contained only traces. Xanthurenic acid has been identified in urine from vitamin B₆-deficient subjects (10,11). It has been called an abnormal metabolite in human urine, however, Price and Dodge (12) were able to identify xanthurenic acid along with kynurenic acid and the 8-methyl ether of xanthurenic acid in normal human urine.

Of current interest in the metabolism of tryptophan is the possibility that certain of its metabolites may cause bladder cancer in man. The chemical similarities between the known bladder carcinogens and tryptophan metabolites are apparent, in that several of the metabolites of tryptophan normally found in urine are aromatic amines and aminophenols. Reports from England indicate that 3-hydroxyanthranilic acid, 3-hydroxykynurenine, 2-amino-3-hydroxyacetophenone and xanthurenic acid 8-methyl ether were weakly carcinogenic in the bladders of mice. Analyses of the urine of bladder tumor patients has shown that only half of 41 patients studied had abnormal quantities of certain tryptophan metabolites, chiefly kynurenine, acetylkynurenine, hydroxykynurenine and kynurenic acid. No definite correlation could be made between the degree of abnormal tryptophan metabolism and type or number of tumors, recurrence rate, age at onset or other clinical findings. Studies on patients with other forms of cancer or with a variety of other non-neoplastic diseases, indicated that many of these subjects had abnormal tryptophan metabolism and that there were a number of clinical conditions, including bladder cancer, in which disorders of tryptophan metabolism occurred (). At the present time the significance of tryptophan metabolism in neoplastic diseases is not clear. The quantity of ingested tryptophan which is metabolized through the kynurenine pathway in man has not been adequately studied. In animals various isotope experiments have indicated that very signifi-

cant amounts of tryptophan, kynurenine and hydroxyanthranilic acid are metabolized to carbon dioxide (14, 15, 16) through the breakdown of 3-hydroxyanthranilic acid (17). That a large amount of ingested tryptophan may also enter this pathway in man is suggested by quantitative studies of the urinary excretion of tryptophan metabolites by subjects who excrete elevated amounts of metabolites.

In rats 46 per cent of tryptophan-2-C¹⁴ is excreted in C¹⁴O₂ and 12 per cent in the urine in a 24-hour period. The remainder of the activity is bound in proteins in the body organs, in enzymes, and in the various retained metabolites.

The proposed study will determine the percentage conversion of labeled tryptophan into C¹⁴O₂, and tryptophan metabolites, as measured by urinary excretion, also the percentage incorporation of tryptophan and its metabolites into blood fractions and other body components, such as coenzyme I. These mechanisms will be studied in all types of neoplastic disease patients. It is planned that a dose of 50 µc of carbon-14-labeled tryptophan-2-C¹⁴ or tryptophan-7a-C¹⁴ will be given orally in a gelatin capsule. However, in terminal patients it is possible that a 100 µc dose may be advisable to obtain sufficient activity in the various fractions for measurement.

A simple formula for beta dosimetry, assuming uniform distribution in a volume large in relation to the range of beta particles (18) is

$$d_{\beta} \text{ (day)} = 51.2 C E_{\beta} \text{ rads where } C = \text{concentration of isotope in } \mu\text{c/gm}$$

$$E_{\beta} = \text{average energy per disintegration in MEV}$$

substituting,

$$dB = 51.2 \times \frac{50}{70,000} \times 0.05 = 0.0018 \text{ rads/day.}$$

This dose of 1.8 m rads/day or 0.66 rads/year would be sustained if there were complete retention indefinitely and is therefore a maximum estimate for whole-body dosage. The known metabolism of labeled tryptophan does not suggest any critical organs except the liver, and even in this case the finding of less than 6% of the ingested dose in the whole liver after 24 hours does not indicate any organ to be more critical than the whole body.

The patients will be given tryptophan-2-C¹⁴ with a specific activity of 0.62 mc/mMole. This compound was obtained from Tracerlab and it has no radioactive components other than the C¹⁴.

From the use of 50 µc of C¹⁴ there will be no radiological hazard to attendant personnel. As part of the study the patients' breath will be collected for analysis during the first few hours and continuously evacuated. Blood and urine samples will be collected for analysis in the research laboratories. Any samples sent to the clinical labs on the first day will be marked "radioactive." No monitoring procedure, except in case of accident, and though no isotope accountability is necessary, experimental design will provide such accountability.

Numerous studies of tryptophan metabolism in patients utilizing a 2 gm dose of L-tryptophan have been conducted by Drs. Brown and Price at the University of Wisconsin with no harmful effects to the patients involved (19).

19. Symposium on Tryptophan Metabolism, sponsored by the Am. Chem. Soc., Atlantic City, N.J. Sept. 14, 1959.

References

1. Quarterly Rev. 5, 227 (1951). C.E. Dalgliesh.
2. In Amino Acid Metabolism, p. 282 (1955). A.H. Mehler.
3. J. Biol. Chem. 182, 679 (1950). H.P. Sarett and G.A. Goldsmith.
4. J. Clin. Invest. 31, 533 (1952). G.A. Goldsmith, H.P. Sarett, U.D. Register and J. Gibbons.
5. J. Lab. Clin. Med. 34, 409 (1949). R.W. Vilter, J.M. Mueller and W.D. Dean
6. J. Nutrition 66, 587 (1958). V.M. Vivian, M.M. Chaloupka, and Reynolds, M.S.
7. Nature 170, 977 (1952). K. Makino, K. Satoh, T. Feyiki and Kawaguchi.
8. Nature 175, 3855 (1955), Musajo, Henassi and Paupajola.
9. J. Biol. Chem. 196, 185 (1952) L. Musajo, A. Spada, and D. Coppini.
10. Arch. Biochem. & Biophys. 21, 237 (1949). L.D. Greenberg, D.F. Bohr, B. McGrath, J.F. Finehart.
11. Arch. Biochem. & Biophys. 33, 243 (1951). H.S. Glazer, J.F. Mueller, C. Thompson, V.S. Hawkins, R.W. Vilter
12. J. Biol. Chem. 223, 699 (1956). J.M. Price and L.W. Dodge.
13. Brit. Med. Bull. 14, 146 (1958). G.M. Bonser, D.B. Clayson, J.W. Jull.
14. J. Biol. Chem. 222, 1069 (1956). L.M. Henderson and L.V. Hankes.
15. Proc. Soc. Exp. Biol. Med. 97, 568 (1958). L.V. Hankes and I.H. Segal.
16. J. Biol. Chem. 225, 349 (1957). L.V. Hankes and L.M. Henderson.
17. J. Biol. Chem. 235, 132 (1960). R.K. Gholson, L.V. Hankes and L.M. Henderson.
18. Radioactive Isotopes in Clinical Practice, p. 98 (1958). E.W. Quinby, S. Feitelberg and S. Silver.

Extra Copy

The Committee on Clinical Investigations and Use of Radioisotopes hereby approves the program with the following title:

Metabolism of Tryptophan

CIRC # 11a has been assigned to this program.

George C. Cotzias

George C. Cotzias, M.D., Chairman

Lewis M. Schiffer

Lewis M. Schiffer, M.D.

Herbert Savel

Herbert Savel, M.D.

Knud Knudsen

Knud Knudsen, M.D.

H.L. Atkins

H.L. Atkins, Consultant

Date: _____

Approval recommended Date _____

Place: Medical Research Center
Brookhaven National Laboratory
Upton, New York 11973

Disapproval _____ Date _____

V. P. Bond
V. P. Bond, M.D.
Chairman, Medical Department

1179816

FORM FOR INITIATION OR REVIEW OF CLINICAL
INVESTIGATIVE PROGRAMS

(Submit original only to Department Chairman)

- A. Title of the proposal: Metabolism of Tryptophan
- B. Sponsoring physician(s): V. P. Bond, E. P. Cronkite and G. Cotzias
- C. Responsible investigator(s): L.V. Hanks, Ph.D., P. Papavasiliou, M.D., R. R.)
Ph.D., (Wisconsin), and J. Bateman, M.D.
- D. Brief description of the study, including its general goals and purpose, and pertinent information on past studies: (Attach additional sheets if necessary)

The tryptophan metabolism study which was initiated under project proposals H-69 and H-69 modified will be expanded to include the study of Parkinsons disease patients.

D and E are answered in the attached sheets.

- E. Reasons why the investigation(s) are to be performed on human subjects.

- F. Type of patient in which the study is to be done (including approximate number of subjects, if known; special restrictions or requirements; method of obtaining consent; etc.):

| | | | | |
|------------------|----|---------------|-----|----------|
| Multiple myeloma | -- | approximately | ten | patients |
| Scleroderma | -- | " | " | " |
| Anemia | -- | " | " | " |
| Parkinsons | -- | " | " | " |

1179817

- G. 1. Are drugs not in the U. S. Pharmacopoeia (USP) or the NNR being used or contemplated for use? Yes ___ No x
2. Is an unusual use of a drug(s) accepted by the USP or NNR contemplated? (An example would be the use of an accepted drug in dosages far exceeding the recommended limits or for purposes distinctly different from the usual indications cited.) Yes ___ No x
3. Are any biological products to be administered that do not bear on their containers or labels notation of approval by the Biological Control Division of the National Institutes of Health? Yes ___ No x
4. Is external or internal radiation other than accepted diagnostic or therapeutic procedures to be administered? Yes ___ No x
5. Are any (other) unusual procedures being performed or proposed which in your judgment may entail a special hazard - particularly a hazard above and beyond any imposed by accepted diagnostic and therapeutic measures for that patient? Yes ___ No x
6. Are any radioisotopes to be administered to human beings? Yes x No ___
- a. If yes, are the radioisotopes to be used solely within the limits of procedures, specifically described in the USP? Yes ___ No ___
Describe the radioisotopic preparation(s):
- b. Or are the radioisotopes to be used only in accordance with a project previously approved by the former Radioisotope Committee of this Department? Yes x No ___
Note the project number: H-69, H-69 (modified) and H-70

IF ANY OF QUESTIONS 1 THROUGH 5 ARE ANSWERED AFFIRMATIVELY, a detailed analysis of the potential hazards must be appended, including pertinent bibliographic citations and other relevant information.

IF QUESTION 6 IS ANSWERED AFFIRMATIVELY, a completed supplementary form for Radioisotope Administration to Human Beings must be appended. However, this form need not be filed provided that question 6a or 6b is also answered affirmatively. A separate form must be submitted for each radioisotopic species to be administered.

James C. Coble
Sponsoring Physician

Committee on Clinical Investigations and
Uses of Radioisotopes

Approval recommended _____ Date _____

Disapproval _____ Date _____

1179818

V. P. Bond, M. D.
Chairman, Medical Department

6/25/63

mlk

Tryptophan, a widely distributed naturally occurring essential amino acid, has been the subject of a great deal of biochemical investigation. It is converted to many other compounds of biological significance, some of which no longer retain the indole nucleus. This amino acid is now recognized as the precursor of such diverse compounds as quinolinic acid, nicotinic acid, xanthomatin, xanthurenic acid, serotonin, Col, and many others. Many points concerning the biosynthesis of these biologically important compounds are still obscure, i.e. Tryptophan balance data on growing animals and the distinct requirement for tryptophan for maintenance in the adult, indicates that a fair amount of carbon from this amino acid is disposed of through the lung and kidney each day. Only a small part of the injected indole nucleus is accounted for in the various excretion products of normal mammals. Tryptophan, like other essential amino acids, must be disposed of by a major route leading to aliphatic compounds and finally to CO₂. There is evidence that tryptophan is converted to niacin in man.² Niacin deficiency in man has been specifically cured by niacin or tryptophan (3-5) and blood pyridinenucleotides were restored to normal levels by supplements of tryptophan after depletion on niacin-deficient diets (6). A number of intermediary metabolites of tryptophan have been isolated or identified in human urines. Makino and coworkers reported that 3-hydroxykynurenine was responsible for the positive diazo reaction frequently observed in severe tuberculosis (7). Musajo, Benassi and Paupajola (8) isolated kynurenine and 3-hydroxykynurenine from urine of patients with various pathological conditions but could not isolate these compounds from normal human urine. Tuberculosis patients studied by Musajo, Spada and Coppini (9) excreted 3-hydroxyanthranilic acid in significant amounts whereas normal urine contained only traces. Xanthurenic acid has been identified in urine from vitamin B₆-deficient subjects (10,11). It has been called an abnormal metabolite in human urine, however. Price and Dodge (12) were able to identify xanthurenic acid along with kynurenic acid and the 8-methyl ether of xanthurenic acid in normal human urine.

Of current interest in the metabolism of tryptophan is the possibility that certain of its metabolites may cause bladder cancer in man. The chemical similarities between the known bladder carcinogens and tryptophan metabolites are apparent, in that several of the metabolites of tryptophan normally found in urine are aromatic amines and aminophenols. Reports from England indicate that 3-hydroxyanthranilic acid, 3-hydroxykynurenine, 2-amino-3-hydroxyacetophenone and xanthurenic acid 8-methyl ether were weakly carcinogenic in the bladders of mice. Analyses of the urine of bladder tumor patients has shown that only half of 41 patients studied had abnormal quantities of certain tryptophan metabolites, chiefly kynurenine, acetylkynurenine, hydroxykynurenine and kynurenic acid. No definite correlation could be made between the degree of abnormal tryptophan metabolism and type or number of tumors, recurrence rate, age at onset or other clinical findings. Studies on patients with other forms of cancer or with a variety of other non-neoplastic diseases, indicated that many of these subjects had abnormal tryptophan metabolism and that there were a number of clinical conditions, including bladder cancer, in which disorders of tryptophan metabolism occurred (13). At the present time the significance of tryptophan metabolism in neoplastic diseases is not clear. The quantity of ingested tryptophan which is metabolized through the kynurenine pathway in man has not been adequately studied. In animals various isotope experiments have indicated that very signifi-

cant amounts of tryptophan, kynurenine and hydroxyanthranilic acid are metabolized to carbon dioxide (14, 15, 16) through the breakdown of 3-hydroxyanthranilic acid (17). That a large amount of ingested tryptophan may also enter this pathway in man is suggested by quantitative studies of the urinary excretion of tryptophan metabolites by subjects who excrete elevated amounts of metabolites.

In Parkinsonian disease, it has been observed that the administration of large doses of dopa will, in 65 per cent of the cases, reverse the neurological symptoms. The 5-hydroxyindole acetic acid (tryptophan metabolite) levels in the urine of these patients are very low. This suggests a malfunction in the metabolism of tryptophan in this disease. The study of the excretion of labeled tryptophan as $C^{14}O_2$ and urinary metabolites before and after dopa treatment may shed some light on some of the problems in the metabolism of these patients.

In rats 46 per cent of tryptophan-2- C^{14} is excreted in $C^{14}O_2$ and 12 per cent in the urine in a 24-hour period. The remainder of the activity is bound in proteins in the body organs, in enzymes, and in the various retained metabolites.

The proposed study will determine the percentage conversion of labeled tryptophan into $C^{14}O_2$, and tryptophan metabolites, as measured by urinary excretion, also the percentage incorporation of tryptophan and its metabolites into blood fractions and other body components, such as coenzyme I. These mechanisms will be studied in all types of neoplastic disease patients and those with scleroderma and Parkinsons disease. It is planned that a dose of 25 to 50 μc of carbon-14-labeled tryptophan-2- C^{14} or tryptophan-7a- C^{14} will be given orally in a gelatin capsule. However, in terminal patients it is possible that a 100 μc dose may be advisable to obtain sufficient activity in the various fractions for measurement.

A simple formula for beta dosimetry, assuming uniform distribution in a volume large in relation to the range of beta particles (18) is

$$d_{\beta} \text{ (day)} = 51.2 C E_{\beta} \text{ rads where } C = \text{concentration of isotope in } \mu c/gm$$

$$E_{\beta} = \text{average energy per disintegration in MEV}$$

substituting,

$$dB = 51.2 \times \frac{50}{70,000} \times 0.05 = 0.0018 \text{ rads/day.}$$

This dose of 1.8 m rads/day or 0.66 rads/year would be sustained if there were complete retention indefinitely and is therefore a maximum estimate for whole-body dosage. The known metabolism of labeled tryptophan does not suggest any critical organs except the liver, and even in this case the finding of less than 6% of the ingested dose in the whole liver after 24 hours does not indicate any organ to be more critical than the whole body.

The patients will be given L-tryptophan-7a- C^{14} with a specific activity of 0.49 mc/mMole. This compound was obtained from Tracerlab and it has no radioactive components other than the C^{14} .

Metabolism of tryptophan - Hankes et al

From the use of 25 to 50 μc of C^{14} there will be no radiological hazard to attendant personnel. As part of the study the patients' breath will be collected for analysis during the first few hours and continuously evacuated. Blood and urine samples will be collected for analysis in the research laboratories. Any samples sent to the clinical labs on the first day will be marked "radioactive". No monitoring procedure, except in case of accident, and though no isotope accountability is necessary, experimental design will provide such accountability.

The dosage of tryptophan given will represent 2.01 to 2.02 gms. of L-tryptophan. This dose has been used routinely in this and other laboratories (19). The pills of L-tryptophan used were prepared from pure L-tryptophan by Abbott Laboratories.

An outline of the study is attached.

1179821

Metabolism of Tryptophan - Hankes et al

References

1. Quarterly Rev. 5, 227 (1951). C. E. Dalgliesh.
2. In Amino Acid Metabolism, p. 282 (1955). A. H. Mehler.
3. J. Biol. Chem. 182, 679 (1950). H. P. Sarett and G. A. Goldsmith.
4. J. Clin. Invest. 31, 533 (1952). G. A. Goldsmith, H. P. Sarett, U.D. Register and J. Gibbons.
5. J. Lab. Clin. Med. 34, 409 (1949). R. W. Vilter, J. M. Mueller and W.D. Dean.
6. J. Nutrition 66, 587 (1958). V. M. Vivian, M. M. Chaloupka, and M. S. Reynolds.
7. Nature 170, 977 (1952). K. Makino, K. Satoh, T. Feyiki and Kawaguchi.
8. Nature 175, 855 (1955). Musajo, Henassi and Paupajola.
9. J. Biol. Chem. 196, 185 (1952). L. Musajo, A. Spada, and D. Coppini.
10. Arch. Biochem. and Biophys. 21, 237 (1949). L. D. Greenberg, D. F. Bohr, B. McGrath, J. F. Finehart.
11. Arch. Biochem. and Biophys. 33, 243 (1951). H. S. Glazer, J. F. Mueller, C. Thompson, V. S. Hawkins, R. W. Vilter.
12. J. Biol. Chem. 223, 699 (1956). J. M. Price and L. W. Dodge.
13. Brit. Med. Bull. 14, 146 (1958). G. M. Bonser, D. B. Clayson, J. W. Jull.
14. J. Biol. Chem. 222, 1069 (1956). L. M. Henderson and L. V. Hankes.
15. Proc. Soc. Exp. Biol. Med. 97, 568 (1958). L. V. Hankes and L. H. Segal.
16. J. Biol. Chem. 225, 349 (1957). L. V. Hankes and L. M. Henderson.
17. J. Biol. Chem. 235, 132 (1960). R. K. Gholson, L. V. Hankes and L. M. Henderson.
18. Radioactive Isotopes in Clinical Practice, p. 98 (1958). E. W. Quinby, S. Feitelberg and S. Silver.
19. J. Lab. and Clin. Med. 69, 313 (1967), L. V. Hankes, R. R. Brown, S. Lippincott, and M. Schmaeler.

Jan 27,

Aim: To survey tryptophan metabolism in patients with Parkinson's Disease, particularly as affected by DOPA treatment.

Experimental: Patients: 3-4 patients known to benefit from DOPA treatment, 2-3 patients who do not benefit from DOPA. Patients maintained 4-5 days on standard control diet and free of drugs, then administer tracer dose of L-7a-C¹⁴-tryptophan with 2.0 gm of L-tryptophan. Collect 24 hr urines one day before and for 2 days after C¹⁴. Collect respiratory C¹⁴O₂ and bloods at intervals. After 1-2 months, the above tryptophan study is repeated during course of therapy with DOPA.

- Analyses:**
- 1) Rates of C¹⁴O₂ release from tryptophan.
 - 2) Analyses for urinary tryptophan metabolites and isolation of them by carrier methods.
 - a) 5-hydroxyindolacetic acid
 - b) 3-hydroxyanthranilic acid
 - c) Kynurenine
 - d) 3-hydroxykynurenine
 - e) Kynurenic acid
 - f) Xanthurenic acid
 - g) O-aminohippuric acid
 - h) N¹-methylnicotinamide
 - i) Quinolinic acid
 - j) Picolinic acid
 - k) Nicotinic acid

Analyses: (continued)

3) Urinary and plasma amino acids by amino acid analyzer.

Note: Draw heparinized blood and take off plasma. Save cells and wash as per previous studies.

Drs. Hanks and Brown

2/21/67

Dr. L.V. Hanks

Clinical Invest. Committee

H-69 (CIRC 11)

The Committee has approved in principle your request dated February 16, 1967. However, doses of L-tryptophan have not been specified. It would be very helpful if you would fill out the attached forms and return them to Dr. Cotzias.

1179825

2/21/67 approved

V. P. Bond, M.D.
CIRC - 11a
(H-69)

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: February 16, 1967

TO: Chairman, Committee on Clinical
Investigations and Uses of Isotopes
FROM: L. V. Hankes, Ph.D.

SUBJECT: Extension of Projects H-69 and H-69
(Modified) to include Parkinsons
Disease

In Parkinsonian disease, it has been observed that the administration of large doses of dopa will, in approximately 60 per cent of the cases, reverse the neurological symptoms. The 5-hydroxyindole acetic acid (tryptophan metabolite) levels in the urine of these patients are very low. This suggests a malfunction in the metabolism of tryptophan in this disease. The study of the excretion of labeled tryptophan as $C^{14}O_2$ and urinary metabolites before and after dopa treatment may shed some light on some of the problems in the metabolism of these patients.

The tryptophan metabolism study which was initiated under project proposals H-69 and H-69(modified) will be expanded to include the study of Parkinsons disease patients.

The patients will be given L-tryptophan-7a- C^{14} with a specific activity of 0.49 mc/mMole. This compound was obtained from Tracerlab as DL-tryptophan-7a- C^{14} and it has no radioactive components other than the C^{14} .

An outline of the study is enclosed.

Sponsoring physician will be G. Cotzias, M.D.

Responsible investigators will be L. V. Hankes, Ph.D., P. Papavasiliou, M.D., and R. R. Brown, Ph.D. (Wisconsin)

Papers published under projects H-69 and H-69 (modified) are listed below:

1. L.V. Hankes, M. Schmaeler and K. Rai, O-Aminophenol: A Urinary Product of Tryptophan Metabolism in the Human, Proc. of the Soc. for Exptl. Biol. and Med., 110, 420 (1962).
2. L.V. Hankes, R.R. Brown, M. Schmaeler, and S. Lippincott, Metabolism of 3-Hydroxyanthranilic Acid-Carboxyl- C^{14} in the Human, Proc. of the Soc. for Exptl. Biol. and Med., 115, 1083 (1964).
3. L.V. Hankes, R.R. Brown, S. Lippincott, and M. Schmaeler, Effects of L-tryptophan Load on the Metabolism of Tryptophan-2- C^{14} in Man, J. Lab. and Clin. Med, Feb. 1967, pg. 313.

1179826

Aim: To survey tryptophan metabolism in patients with Parkinson's Disease, particularly as affected by DOPA treatment.

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Analyses: (continued)

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Drs. Hanks and Brown

1179828