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

REPLACED BY CIRC # 83
✓

REPOSITORY Records Holding Area, Bldg. 494
COLLECTION Committee-Clinical Investigations and
uses of Radioisotopes
BOX No. 4
FOLDER CIRC # 2, 2a, 2b



The Committee on Clinical Investigations and Use of Radioisotopes

hereby approves the program with the following title:
~~disapproves~~

KINETICS OF HEMOPOIETIC CELL PROLIFERATION STUDIED BY
MEANS OF NUCLEIC ACID LABELING WITH TRITIATED PURINES
AND PYRIMIDINES

CIRC # 2b has been assigned to this program

George C. Cotzias
George C. Cotzias, M.D., Chairman

Knud Knudsen
Knud Knudsen, M.D.

Donald C. Borg
Donald C. Borg, M.D.

Harold L. Atkins
Harold Atkins, M.D.

Lewis M. Schiffer, M.D.

11 June 68
Date

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

Approved: E. P. Cronkite
E. P. Cronkite, M.D.
Chairman, Medical Department

6/07/1968 Date

117935b

3 June 1968

Lewis M. Schiffer, M.D.

LMC
E. P. Cronkite, M.D.Chairman
6/3/68ADDENDUM TO CIRC #2 (2b)

Permission is requested to perform a thoracic duct cannulation upon a patient with chronic lymphocytic leukemia.

The surgical technique is relatively simple and has been performed in human beings and animals for many years. Dr. Arjun D. Chanana, Division of Hematology, has extensive experience in three different species of animals and will perform the surgery.

The patient has a large brain tumor and has had a cerebral vascular accident and cannot, himself, sign a consent form. His wife has agreed and will sign an informed consent form. This is satisfactory to Mr. Rathvon.

We wish to perform this cannulation and thoracic duct-venous shunt in conjunction with the cell labeling studies under which this addendum is appended.

The importance of this project lies in the estimation of cell (lymphocyte) recycling ability from blood → lymph → blood. This is of great theoretical importance in understanding chronic lymphocytic leukemia cell kinetics.

1179357

Minutes CIRC Meeting

8 May 1972

Present: J.S. Robertson, S.H. Cohn, H.R. Connell, R.A. Love, G.A. Price,
N.P. Rathvon, Jr.

The meeting was held in the Small Conference Room of the Medical Research Center and opened by Dr. Robertson at 1400.

The minutes of the previous meeting were accepted as distributed.

The Chairman distributed some copies of "The Institutional Guide to DHEW Policy on Protection of Human Subjects". More copies of the publication have been ordered so that every Committee Member can have a copy.

Dr. Robertson reported on the status of CIRC projects that are on the active list but are overdue for re-review. Of the nine projects in this category, seven will be reclassified as inactive; requests from Dr. Atkins for review of the remaining two are expected. The memorandum to Dr. Cronkite from Dr. Robertson listing the CIRC proposals involved is attached.

The Committee received a communication from Dr. Cotzias requesting that CIRC #58 be made inactive.

The following CIRC proposals were reviewed:

1. CIRC #32, revised. This proposal, "The Study of Polycythemia Vera", was approved as submitted.
2. Annual recertification of CIRC #48 was approved. It was noted that the original form for Initiation or Review of Clinical Investigative Programs was unsigned. The form should be signed by Dr. Shreeve.
3. Annual recertification of CIRC # 63 was approved with the condition that the word radioactive be included on the Consent Form, that is, paragraph 1 on the Consent Form should read: Thyroid uptake with radioactive iodine-123.....
4. CIRC # 82, revised. The proposal was approved subject to clarification on the Consent Form of whether 50 calendar days or 50 samplings is intended. It is suggested the wording on the Consent Form be:
"..... be obtained 3 mornings a week and for a total period of 50 days

The Committee then entered into a discussion of the procedure for recording patient's x-rays and whether the x-rays should be included in the patient's radiatio record. No action was taken in this matter.

The meeting was adjourned at 1615.

Respectfully submitted,


Helen R. Connell

Enc.

Memorandum to Dr. E.P. Cronkite
from Dr. J.S. Robertson

1179358

BROOKHAVEN NATIONAL LABORATORY
MEMORANDUM

DATE: 17 April 1972

TO: E. P. Cronkite
FROM: J. S. Robertson
SUBJECT: C I R C

Reference: CIRC minutes for meeting of 3 April 1972.

As indicated in the referenced minutes, the CIRC is concerned about the continued appearance of certain projects on the active list but which are over two years out of date and for which requests for review have not been received. It is proposed that these now be reclassified as inactive. They can, of course, subsequently be restored to the active list through the usual procedure.

Specifically, it is proposed that the following projects be relegated to the inactive list:

<u>CIRC NO.</u>	<u>Date Orig.</u>	<u>Principal Investigator</u>	<u>Description</u>
2	6/30/64	Dr. Cronkite	H-3 Cell Kinetics
2A	2/28/67	Dr. Cronkite	C14 Labelled Pyrimidine
15	6/30/64	Dr. Atkins	TC-99M Scanning TC04
15A	2/5/65	Dr. Atkins	TC-99M Scanning TC-S
18	6/30/64	Dr. Cronkite	CO-60 ECIB
18A	12/15/64	Dr. Cronkite	CO-60 ECIB leukemia
18B	2/25/65	Dr. Cronkite	CO-60 ECIB pre kidney transplant
18C	7/12/65	Dr. Schiffer	CO-60 ECIB amphotericin B
18D	1/27/69	Dr. Rai	ECIB CLL

JSR:mas

1179359

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: 3/28/72

TITLE: Addendum re: Use of 14C labeled pyrimidines

TO: Dr Cronkite
FROM: R.B. Aronson, Ph.D. RBA
SUBJECT: CIRC Proposal 2A

In compliance with recent FDA and HEW notices requiring periodic reviews of clinical research projects, your CIRC proposal, number 2A is scheduled for review soon. Please indicate at the bottom of the page if this proposal should be continuing or placed on the inactive list.

This proposal was last reviewed and approved by the Committee on 2/28 1967. Do you wish to make any substantive changes in your proposal? yes

Have you noticed any adverse effects during the experimental program which have not already been reported to the Department Chairman's Office? NO. Please include the nature and frequency of such effects.

Approximately how many patients have been submitted to the experimental regime since the last approval? _____

The Sponsoring Physician on this proposal is E.P. Cronkite. Has there been a change of Sponsoring Physician or Responsible Investigators? _____

If you have obtained IND numbers from the FDA in connection with this proposal please list on a separate sheet the compounds and corresponding IND numbers, and attach. *Now IND has been submitted for 3 HTDR*

Please attach to this sheet copies of any reports submitted to the FDA, HEW, or other Granting Agency (in connection with this proposal and the IND numbers given above), since the last CIRC approval date.

Please add any additional information which may be of use to the Committee in its deliberations. Include a copy of the Patient Consent Form now in use for this study.

CIRC PROPOSAL NUMBER _____ IS: Continuing
Inactive

Please combine 2, 2A, H52 & 83 into a single proposal
Signed E.P. Cronkite Date 28 Mar 72

Please return this completed form to Dr. R.B. Aronson as soon as possible.

1179360

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

CIRC # 2a 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.

Date: February 28, 1967

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

Approval recommended _____ Date _____

Disapproval _____ Date _____

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

1179361

Feb. 21, 1967

Dr. P.C. Vincent

G.C. Cotzias, Chrm. Clin.Inves.Comm.

Memo dated Feb. 8 1967 CIRC-2

The question of pyrogenicity of the pyrimidine compounds was discussed by the committee. It was thought that dangers from pyrogens will be minimized by the fact that only pyridimidines incorporated within cells during the incubation will be administered to humans. Therefore, standard tests for pyrogenicity do not seem applicable to this procedure. Further, it appeared that the washings involved will mitigate against the presence of pyrogens in the material finally injected.

The proposal has been approved by the committee.

1179362

BROOKHAVEN NATIONAL LABORATORY

M E M O R A N D U M

DATE: 8 February 1967

TO: Clinical Investigations Committee

FROM: P. C. Vincent, M. D.

SUBJECT: Addendum to project CIRC-2 (H-52)
"Kinetics of Hemopoietic Cell
Proliferation by Means of Nucleic
Acid Labeling with Tritiated
Purines and Pyrimides."

To allow similar studies with ^{14}C labeled pyrimidines. The reasons for performing the investigations in human subjects, and the type of patient to be studied, are given in CIRC-2.

Reason for Using ^{14}C Isotopes:

^{14}C pyrimidines will be incorporated into leukemic cells and/or into other cells in the peripheral blood in vitro in order to follow the survival and fate of these cells in the circulation. This will be done where a similar study has been done using ^3H pyrimidines and where it is desirable to repeat the study. Specifically, for example, we hope to compare the survival of lymphocytes before and after irradiation of the blood (ECIB) in patients with chronic lymphocytic leukemia. ^{14}C labeled cells can be distinguished from ^3H - labeled cells by autoradiography because of the longer particle tracks in the emulsion. Thus a second survival curve could be computed despite the persistence of some ^3H - labeled cells in the circulation.

Proposed Method:

White cell concentrates from the peripheral blood will be incubated in vitro under sterile conditions with ^{14}C pyrimidine at a concentration of between 1 and 2 μc per ml. The maximum anticipated amount used for incubation would be 400 μc and the average would be between 100 and 200 μc . After incubation the cells will be washed twice with a medium containing an excess of nonradioactive pyrimidine and injected intravenously into the patient ~~from~~ whom the cells were derived.

Experience in this laboratory, using ^3H pyrimidines under these conditions, has shown that only 10% of the added isotope remains in the cell fraction. Further it is estimated that more than 90% of the isotope in the cell fraction at the time of injection is actually contained in the cells. Thus the dose of isotope administered to the patient can be estimated as 10% of the amount used in incubation, and the distribution within the body will be that of the injected cells.

1179363

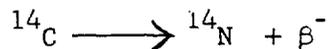
Pharmacology:

The pharmacology of the pyrimidines has been considered in CIRC-2. The ^{14}C compounds are all available as sterile aqueous solutions; the specific activities (Schwarz BioResearch) are as follows:

Cytidine -2 ^{14}C	20-40	$\mu\text{c}/\text{m mole}$
Deoxycytidine -2 ^{14}C	8-15	" "
Deoxyuridine -2 ^{14}C	20-50	" "
Thymidine -2 ^{14}C	6-10	" "
	and >25	" "
Uridine -2 ^{14}C	25-40	" "

Pyrogenicity:

The Schwarz preparations are sterilized by Millipore filtration and are quite unlikely to contain pyrogens.

Dosimetry:

Half-life 5730 years

Maximum energy 0.155 MeV

Mean energy (\bar{E}_β) 0.048 MeV

(1)

An attempt can be made to estimate the radiation exposure both to the labeled cells and to the whole body.

(a) Radiation to labeled cells. Appendix i gives an estimate of the size of this dose. Effects on the labeled cells will not pose any health problem. Radiation death of the labeled cells might disturb the experiment but not the patient, while leukogenic effects can be disregarded in leukemic patients or in patients with other terminal illness.

(b) Whole body exposure.

1) The proposed maximum dose of $40\mu\text{c}$ injected is less than the maximal permissible body burden of $400\mu\text{c}$ (3).

2) If a dose of $40\mu\text{c}$ were completely retained the radiation dose would be:

$$\begin{aligned} & 51.2 \bar{E}_\beta \text{ C rad/day} \quad (1, \text{ p.824}) \\ & = 51.2 \times 0.048 \times \frac{40}{70,000} \text{ rad/day for 70 kg man.} \\ & = 1.404 \times 10^{-3} \text{ rad/day.} \end{aligned}$$

In most instances the administered dose would be between 10 and $20\mu\text{c}$ ^{14}C , so that the β dose rate would correspondingly be between 3.511×10^{-4} rad/day 7.022×10^{-4} rad/day.

3) Alternatively assume an effective half life for lymphocytes of 100 days. Since these cells will contain most of the injected isotope (in cases of chronic lymphocytic leukemia) the cumulative β dose becomes, since,

$$D_{\beta}(\infty) = 73.8 \bar{E}_{\beta} \text{ Co. Teff. rad (1, p828)}$$

for $40\mu\text{c}$ in a 70 kg man.

$$D_{\beta}(\infty) = 73.8 \times 0.048 \times \frac{40}{70,000} \times 100 \text{ rad}$$

$$= 0.202 \text{ rads.}$$

and for $20\mu\text{c}$ in a 70 kg. man $D_{\beta}(\infty) = 0.101 \text{ rads.}$

for $10\mu\text{c}$ in a 70 kg man $D_{\beta}(\infty) = 0.0505 \text{ rads.}$

4) Dose estimates with prior ^3H studies:

Since ^{14}C pyrimidines will be used in patients previously studied with ^3H pyrimidines the total radiation exposure has to be calculated.

$$\text{for } ^3\text{H } \bar{E}_{\beta} = .0055 \text{ MeV (1)}$$

For a maximum of $40\mu\text{c}$ incorporated in the cells injected, in a 70 kg man

$$D_{\beta}(\infty) = 73.8 \times .0055 \times \frac{40}{70,000} \times 100 \text{ rads}$$

$$= .0232 \text{ rads for } ^3\text{H dose.}$$

Total maximum whole body radiation would therefore be, for $40\mu\text{c } ^3\text{H}$ followed by $40\mu\text{c } ^{14}\text{C}$,

$$D_{\beta}(\infty) = .2252 \text{ rads.}$$

5) It should be noted that these estimates are based on an uptake of 10% of the added isotope by the cells (see methods). If this were in error the estimates could be greater by a factor of 10.

Summary: An addendum to CIRC-2 to allow the use of ^{14}C labeled pyrimidines in selected patients is requested. Although in most instances only one study would be undertaken in each patient, permission is also requested to repeat the study if indicated.

1179365

8 February 1967

- References:
- 1) Hine, G. J. and Brownell, G. L.: Radiation Dosimetry, 1963, Academic Press, New York.
 - 2) Bond, V. P. and Feinendegen, L. E.: Intranuclear ³H-Thymidine. Dosimetric, Radiobiological and Radiation Protection Aspects. Health Physics 12, 1007, 1966.
 - 3) National Bureau of Standards Handbook No. 52, 1953 and Handbook No. 69, 1959.

aw

1179366

Calculation of Radiation Dose to Labeled Cells.

This is an attempt to calculate the ^{14}C dose to the labeled cells. The values calculated for ^3H (2) cannot be adapted to ^{14}C , because of the higher energy and greater path length of the latter isotope. Consider cells labeled with ^{14}C with an average grain count after 30 days of 30 grains. Assuming an autoradiographic efficiency of 10%, this is equivalent to:

$$\frac{30}{30} \times \frac{10}{24 \times 60} \quad \text{DPM}$$

For the maximum energy of ^{14}C (0.155 MeV) the maximum β particle range in water (1) can be calculated as 285μ . Assume an average pattern length of 140μ .

The linear energy transfer is thus: $\frac{E_{\beta}}{140} = \frac{.048}{140} \quad \text{Mev per } \mu$.

If each particle originates in the centre of nucleus of radius of 3μ in a cell radius 4μ then each particle transfers to the nucleus

$$\frac{3 \times .048}{140} = \text{Mev}$$

and to cytoplasm $\frac{1 \times .048}{140} \quad \text{Mev}$

and for n DPM, the rate of energy transfer is the product (n x energy transfer from one particle over the given distance) for the nucleus this is:

$$\begin{aligned} & \frac{3 \times 48 \times 10^{-3}}{140 \times 144} \quad \text{Mev per minute} \\ & = \frac{10^{-3}}{140} \quad \text{Mev per minute} \end{aligned}$$

Converting to ergs: $= \frac{10^{-3} \times 1.602 \times 10^{-6}}{140} \quad \text{ergs per minute}$

Assuming unit density this can be converted to rads per minute.

$$\begin{aligned} & = \frac{1.603 \times 10^{-9}}{140 \times \frac{4}{3} \times \pi \times 27 \times 10^{-12} \times 10^2} \quad \text{rads per minute} \\ & = \frac{1.012 \times 10^{-3}}{\quad} \quad \text{rads per minute} \end{aligned}$$

for cytoplasmic shell the rate of energy transfer is

$$\begin{aligned} & \frac{48 \times 10^{-3}}{140 \times 144} \quad \text{Mev per minute} \\ & = \frac{10^{-3}}{520} \quad \text{Mev per Minute} \end{aligned}$$

1179367

Appendix:

-2-

Converting to ergs: $\frac{1.602 \times 10^{-9}}{520}$ ergs per minute

since the volume of the shell is $\frac{4}{3} \pi (4^3 - 3^3)$, assuming unit mass energy

in rads per minute

$$= \frac{1.602 \times 10^{-9}}{520 \times \pi \times \frac{4}{3} \times 37 \times 10^{-12} \times 10^2}$$

$$= \underline{1.99 \times 10^{-4} \text{ rads per minute}}$$

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: 3/28/72

TITLE: Kinetics of Hemopoietic Cell Proliferation Studied by Means of Nucleic Acid Labeling with Tritiated Purines and Pyrimidines including forum

TO: From Dr. Cronkite

FROM: To R.B. Aronson, Ph.D. *RBA*

SUBJECT: CIRC Proposal 2

In compliance with recent FDA and HEW notices requiring periodic reviews of clinical research projects, your CIRC proposal, number 2 is scheduled for review soon. Please indicate at the bottom of the page if this proposal should be continuing or placed on the inactive list.

This proposal was last reviewed and approved by the Committee on 6/30 1964. Do you wish to make any substantive changes in your proposal? yes

Have you noticed any adverse effects during the experimental program which have not already been reported to the Department Chairman's Office? NO. Please include the nature and frequency of such effects.

Approximately how many patients have been submitted to the experimental regime since the last approval? _____

The Sponsoring Physician on this proposal is E.P. Cronkite. Has there been a change of Sponsoring Physician or Responsible Investigators? _____

If you have obtained IND numbers from the FDA in connection with this proposal please list on a separate sheet the compounds and corresponding IND numbers, and attach. *New IND has been submitted for ³HTd R*

Please attach to this sheet copies of any reports submitted to the FDA, HEW, or other Granting Agency (in connection with this proposal and the IND numbers given above), since the last CIRC approval date.

Please add any additional information which may be of use to the Committee in its deliberations. Include a copy of the Patient Consent Form now in use for this study.

Please combine 2, 2A, #52, 783 into a single proposal
CIRC PROPOSAL NUMBER _____ IS: Continuing
Inactive
Signed Eugene P. Cronkite Date 29 Mar 72

Please return this completed form to Dr. R.B. Aronson as soon as possible.

1179369

SPEED LETTER

TO *Dr. Cronkite*

FROM *Dr. Robertson*

SUBJECT *CIRC # 2*

MESSAGE

DATE *17 May 1971*

—FOLD

This proposal was tabled by the committee, which considered the request for renewal deficient in the following respects:

- 1) There is no update information, such as whether any adverse reactions occurred or as to whether the same category of patients is to be studied.*
- 2) The phrase "limited life expectancy" was not considered meaningful.*
- 3) Oral consent is no longer considered to be sufficient.*
- 4) Aronson's memo should be returned for the record.*
- 5) There can be only one sponsoring physician.*

—FOLD

SIGNED *J. Robertson*

REPLY

DATE

19

—FOLD

1179370

HOSPITAL OF MEDICAL RESEARCH CENTER,
BROOKHAVEN NATIONAL LABORATORY
Upton, New York 11973

CLINICAL INVESTIGATION AUTHORIZATION FORM

Purpose of Review: Initial Revision Continuing Addendum

Title:

Kinetics of Hemopoietic Cell Proliferation Studied by Means of Nucleic Acid Labeling with Tritiated Purines and Pyrimidines including forum CIRC 2A-Addendum re: Use of ¹⁴C labeled pyrimidines.

CIRC# 2

Assigned on (date) 8/19/63

To Chairman, CIRC,

The proposal for clinical investigation identified by the above CIRC number and title is forwarded herewith for review and recommendation.

Please substitute A.D. Chanana for T.M. Fliedner as co-investigator

E. P. Cronkite 14 May '71
E.P. Cronkite, M.D., President of Staff Date

To President of Staff,

The Clinical Investigation and Uses of Radiosotopes Committee reviewed the above identified proposal on 17 May 1971 and recommends _____ with the following modifications:

J.S. Robertson, Chairman

G.C. Cotzias, Alt. Chairman

H.R. Connell

S.H. Cohn

E.A. Popenoe, Alternate

R.A. Love

G. Price

J.F. Klopfer

N.P. Rathvon, Jr.

S.E. Duby, Alternate

A.P. Wolf, Alternate

To _____,

The above titled and numbered proposal is _____ subject to the following:

E.P. Cronkite, M.D., President of Staff Date

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: May 20, 1971

TO: DR. E.P. Cronkite

FROM: R.B. Aronson, Ph.D.

SUBJECT: CIRC Proposal 2

In compliance with recent FDA and HEW notices requiring periodic reviews of clinical research projects, your CIRC proposal, number 2 is scheduled for review soon. Please indicate at the bottom of the page if this proposal should be continuing or placed on the inactive list.

This proposal was last reviewed and approved by the Committee on NOVEMBER 8 1963. Do you wish to make any substantive changes in your proposal? _____

Have you noticed any adverse effects during the experimental program which have not already been reported to the Department Chairman's Office? _____. Please include the nature and frequency of such effects.

Approximately how many patients have been submitted to the experimental regime since the last approval? _____

The Sponsoring Physician on this proposal is _____. Has there been a change of Sponsoring Physician or Responsible Investigators? _____

If you have obtained IND numbers from the FDA in connection with this proposal please list on a separate sheet the compounds and corresponding IND numbers, and attach.

Please attach to this sheet copies of any reports submitted to the FDA, HEW, or other Granting Agency (in connection with this proposal and the IND numbers given above), since the last CIRC approval date.

Please add any additional information which may be of use to the Committee in its deliberations. Include a copy of the Patient Consent Form now in use for this study.

CIRC PROPOSAL NUMBER 2 IS: Continuing
Inactive

Signed _____ Date _____

Please return this completed form to Dr. R.B. Aronson as soon as possible.

1179372

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

Kinetics of Hemopoietic Cell Proliferation Studied by
Means of Nucleic Acid Labeling with Tritiated Purines
and Pyrimidines

CIRC # 2 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

1179373

FORM FOR INITIATION OR REVIEW OF CLINICAL
INVESTIGATIVE PROGRAMS

13

(Submit original only to Department Chairman)

- A. Title of the proposal: KINETICS OF HEMOPOIETIC CELL PROLIFERATION STUDIED BY MEANS OF NUCLEIC ACID LABELING WITH TRITIATED PURINES AND PYRIMIDINES.
- B. Sponsoring physician(s): E.P. Cronkite and T. M. Fliedner
- C. Responsible investigator(s): E. P. Cronkite and T. M. Fliedner
- D. Brief description of the study, including its general goals and purpose, and pertinent information on past studies: (Attach additional sheets if necessary.)
(Please see attached sheet)

- E. Reasons why the investigation(s) are to be performed on human subjects.
It is the purpose of this study to investigate the proliferative kinetics of blood cells in man in steady-state equilibrium and in proliferative disorders. Human diseases of proliferation are distinctly different from those in animals since the clinical course, the time parameters of human hemopoiesis are markedly different from animals. Therefore, an understanding of hemopoietic disorders in man, will be possible only through a study of suitable patients.
- 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.): The studies proposed will include only patients at an age where procreation is impossible and/or in diseases which have a limited life expectancy. (Continued on separate sheet)

1179374

- G. 1. Are drugs not in the U. S. Pharmacopoeia (USP) or the NNR being used or contemplated for use? Yes X No
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 X No
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 X
- 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-52

(See separate sheet for Item G.)

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.

Eugene P. Cronkite
Sponsoring Physician

Committee on Clinical Investigations and
Uses of Radioisotopes

Approval recommended ✓ Date 11/8/63

Disapproval Date

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

JUN 29 1964

1179375

6/25/63
mlk

GENERAL BACKGROUND: The dynamics of the hemopoietic equilibrium between cell formation and destruction and its disturbances in man have been of interest to clinical investigators for decades. However, until radioactive isotopes became available recently, there were no methods to study these factors quantitatively in man.

The use of radioactive iron and phosphorus opened new avenues for the investigation of the kinetics of hemopoietic cell proliferation. However, these approaches did not permit a direct study of the proliferative kinetics of the actual processes of formation, proliferation, maturation and migration at the cellular level.

The synthesis of tritium labeled thymidine as a specific DNA marker by Hughes in 1956 opened another approach to these problems. Thymidine is incorporated into newly synthesized DNA of cells capable of cell proliferation. It is stable and only diluted by cell division. The tritium labeling thus permits a stable labeling of cells which can be observed and quantitated by high resolution single cell autoradiography. Information about kinetics of cellular proliferation can be obtained by observing progression of label through mitosis, the changes of the labeling intensity and/or the fraction of labeled cells of a particular cell type as a function of time. Since then, a number of other purines and pyrimidines have been labeled with tritium and can be used to study various aspects of the process of hemopoiesis at a cellular level.

OUTLINE OF PROPOSED STUDY: It is the purpose of the proposed investigation to study the kinetics of hemopoietic cell renewal in various clinical conditions by means of nucleic acid labeling techniques using tritiated pyrimidines and purines.

Suitable patients will be given intravenously tritiated pyrimidines and purines in doses up to $0.2\mu\text{c}/\text{gm}$ of body weight per single injection or up to $1\mu\text{c}/\text{gm}$ of body weight total per continuous infusion for several hours in leukemics only.

In another type of study, blood cells will be removed from the patient, incubated in vitro with a tritiated pyrimidine or purine and reinfused in order to study the fate of the labeled cells and/or of the labeled material.

Serial bone marrow and blood samples (in selected cases lymphnode tissue) as well as saline mouth wash preparations will be obtained and processed for autoradiography. After exposure, developing, fixation, washing and staining, these samples will be evaluated microscopically.

Additional sheet for Item F.

a) Study of hemopoiesis in a "steady state". Patients with malignant disorders not involving the hemopoietic system (brain tumors, solid cancer).

b) Study of proliferative diseases of hemopoiesis.

1. Leukemia: acute and chronic myelocytic and lymphocytic leukemias (aleukemic or leukemic).

2. Multiple myeloma

3. Anemia: Vitamin deficiency (such as pernicious anemia) or responsive anemias, acquired or congenital hemolytic anemias

4. Leukopenia: Various forms of "splenic neutropenia" in particular "Felty's syndrome"

5. Thrombocytopenia: Various forms, in particular idiopathic thrombocytopenia purpura before and after splenectomy.

Although only one administration of a tritium labeled purine or pyrimidine will be the rule, a repeated study will be desirable in all those cases in which it was possible to correct the proliferative defect ("splenic^{ic} neutropenia" before and after splenectomy, acute leukemia in relapse and remission, pernicious anemia before and after treatment).

In all instances, oral consent will be obtained from patients after a suitable explanation of purpose and extent of study.

1179377

Additional sheet for Item G

Although thymidine and cytidine are not in the USP, extensive pharmacologic studies have been performed utilizing chemical amounts measured in hundred of milligrams without any detectable effects. Thymidine has been used as a partially effective therapy of pernicious anemia in gram amounts without any side effects. Only fractional milligram amounts of radio thymidine or cytidine are used.

The radiation toxicity is considered under H-52 (radioisotopic approved project.)

To date, 19 patients have been given tritiated thymidine at dose levels up to 0.19 μc /gram body weight without any known harmful effects.

1179378

Investigators: E.P. Cronkite

Title: Use of tritiated pyrimidines in patients in addition to tritiated thymidine.

Date: July 30, 1962

Project: H-52

Approved: Proviso: That administration ^{be restricted to} of 1 dose per person until the number of doses per person are clarified by Dr. Cronkite. Committee on Use of Radioactive Isotopes in Patients:

George C. Cotzias.
George C. Cotzias, M. D.
Chairman

E. Schackow.
E. Schackow, M.D.

Lewis M. Schiffer
L. M. Schiffer, M. D.

V. P. Bond
V. P. Bond, M. D. (Ex-Officio)

J. S. Robertson
J. S. Robertson, M. D.
(Ex-Officio)

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: 30 July 1962

TO: Chairman, Radioisotope Committee
George Cotzias, M. D.
FROM: Eugene P. Cronkite, M. D.

SUBJECT: Use of tritiated pyrimidines in patients in addition to tritiated thymidine. (Refer to isotope authorization H-52.)

1. Tritiated cytidine, deoxyuridine, uridine and other tritiated pyrimidines are precursors for both DNA and RNA. Extensive studies have been performed with these on rats without any detectible evidence of injury at levels up to $1 \mu\text{c}/\text{gm}$ of body weight. With tritiated thymidine, the studies are rather clearcut. It is necessary to give doses in excess of $1 \mu\text{c}/\text{gm}$ to obtain any detectible radiation injury. Between 1 and $5 \mu\text{c}/\text{gm}$ there is negligible effect, with doses of $20 \mu\text{c}/\text{gm}$ in a mouse, the effect is approximately equal to that produced by 50 to 75 rads of external radiation when measured with the testicle and the lymph nodes. The evidence for radiation injury has been measured by the decrease in the number of spermatogonia (Johnson and Cronkite, reprint attached) and by the appearance of pyknotic lymphocytes in the lymph nodes.

2. To date the tritiated pyrimidines with the exception of thymidine have not been administered to man. The problems concerned with the computation of dose are similar to those expressed in H-52.

3. There is some evidence that the leukemic cell may have a metabolic block against the incorporation of thymidine into DNA and that tritiated deoxyuridine or cytidine may label more satisfactorily. These observations are based upon in vitro studies. The evidence for the former is based on studies by Dr. Feinendegen in this Laboratory as yet unpublished. Since this is a key problem in the interpretation of the kinetics of cell proliferation in malignant, it is requested that we be given authority to administer tritiated pyrimidines other than thymidine in doses up to $0.2 \mu\text{c}/\text{gm}$ of body weight. The only patients for which this authority is requested are patients with leukemia, multiple myeloma, other malignant blood dyscrasias or solid cancers that may become available for study. It will be limited to individuals in whom there is no likelihood of procreation and in whom life expectancy is limited. In all of these situations it is noted that radiation therapy is an acceptable and at time a very satisfactory treatment. The amounts of radioactive material given as compared to the amounts of radioactive phosphorus that would be used therapeutically and which also is incorporated into RNA and DNA is very small indeed. Single doses of radioactive phosphorus up to 6 mc are acceptable means of therapy in the leukemic states. The rad

1179380

30 July 1962

dose from this amount of material averaged throughout the body is of the order of 100 rad and in the target tissue may give an integral dose in excess of 200-300 rad depending upon how the dose is computed. As a matter of fact, these dose computations are in reality futile since one does not have enough information on the microscopic distribution of the isotope.

If one considers the pyrimidine activity exclusively as if it were tritium oxide and assuming uniform distribution with a biological half-life of 19 days, the infinite whole-body dose to a 50 kilogram individual is 0.18 rep following the injection of 1 μc . With the present request, it is conceivable that ten times this amount of material would be administered into such an individual resulting in a total body dose of 1.8 rep.

Whereas it is clear that the dangers of tritiated pyrimidines in terms of potential carcinogenic effect and genetic hazards have not been evaluated adequately as yet, it is clear that acute effects from the doses that are needed for adequate autoradiography are not detectible. Acute effects after large doses are minimal to nondetectible. One can compute doses to the bone marrow cells as follows. The volume of bone marrow nuclei assuming spheres will vary from 100 cu. microns (radius about 3 microns to 1300 cu. microns with radius of about 7 microns). The volume of the cells that perpetuate hemopoiesis is not certain but probably varies between 200 and 500 cu. microns. If one uses Hughes estimate of 30 r to a 200 micron nucleus from 60 disintegrations, one can estimate doses to various primitive hemopoietic cells. Grain counts over the larger nuclei on 30 days exposure are as high as 90 after a dose of .1 $\mu\text{c}/\text{gm}$ to patients. If one assumes an autoradiographic efficiency of 1% there are about 300 disintegrations per day. If the volume is the same, the average maximum dose to the nucleus will be of the order of 150 r the first day. Since cell division in these cells occurs every 24 hours or even more rapidly in some cases, the dose is rapidly cut into half for subsequent time periods. In the above computation, dose was estimated on maximum grain count and a low autoradiographic efficiency factor which overestimates the dose. For the first seven days (assuming 7 mitosis) the maximum nuclear dose would be of the order of 300 r. On the basis of mean grain counts it would be of the order of 70 to 100 r during the first week for the primitive progenitor cells as defined by us on cytologic grounds. It is quite evident that the average dose varies with autoradiographic efficiency and nuclear volume of the cell which perpetuates hemopoiesis but which is not identified with certainty. Since neither is known, these numbers have little real meaning. Lastly, the average dose has no real meaning since only the dose to vulnerable structures is of significance in determining the effect. There is no doubt that tritiated thymidine does produce effects but these effects must be determined by empiric studies, not by computation. Apropos of this, there have been published recently, (Nature, vol. 193, pp. 705-706, 1962) data that shows mutations can be induced in the mouse with tritiated thymidine if one uses quite high doses repeatedly. In these studies high doses were used 300 μc spread out into 6 fractions over 2 days. This gave uniform labeling of a wide band of spermatozoa and when these sperm were used for fertilization there was a distinct increase in the incidence of

1179381

30 July 1962

of dominant lethal mutations as determined by killing the pregnant females and examining the fetuses early. Thus, it is proved that mutations can be produced by thymidine. In the case of tritiated pyrimidines that go into both RNA and DNA with relatively the same efficiency as thymidine, the preceding maximal dose estimates will also pertain. However, these dose estimates are of the order that would be used therapeutically for blood dyscrasias and from this standpoint are not clinically contraindicated. Another modifying factor is the implication of the Taylor, Wood, Hughes chromosome replication model on dose. Since the chromatids behave with the exception of the perturbation by crossing over as individual units, the labeled chromosomes will be disposed of in successive divisions according to a binomial distribution. With a chromosomal number of 46 as pertains to man and thus after the fifth division the number of cells having no label will progressively increase. (Cronkite et al, Nature, vol. 189, pp. 153-154, January 14, 1961)

It is true that Painter et al have shown inhibition of cell division with doses as low as 0.02 $\mu\text{c}/\text{ml}$ of culture medium. However, these results can not be carried directly to the mammal, in which material is given intravenously, because the availability times are widely different in the two situations.

Admittedly, it is not feasible to give realistic estimates of the doses of radiation to any of the critical target tissues. However, inasmuch as radiation is a recognized therapeutic medium in the diseases discussed and since there is no empiric evidence of tangible injury at the levels considered it is requested that permission be granted to administer up to 0.2 $\mu\text{c}/\text{gm}$ of body weight to individuals with short life expectancy as a result of malignant blood dyscrasias or other malignant disease and in whom procreation is highly unlikely.

BPC:aw

1179382

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: 24 July 1962

TO: Radioisotope Committee

FROM: Eugene P. Cronkite, M. D. *EPC*SUBJECT: Addendum to project H-52
(H³thymidine)

H³ thymidine has been used in patients with neoplastic diseases or various hemopoietic disorders to study the life cycle of various blood cell systems. Through in vivo and in vitro studies, it has been found that only a small fraction of blood lymphocytes and tissue lymphocytes are labeled by means of tritiated thymidine, due to its exclusive incorporation into newly synthesized DNA. Up to 19mc of tritiated thymidine have been administered to patients without demonstrable somatic effects on lymphocytic or myelocytic tissue (approximately 0.3 $\mu\text{c}/\text{gm}$ body weight). In animal studies performed in this laboratory, somatic effects are suggested at doses of 1 $\mu\text{c}/\text{gm}$ and are demonstrable with doses of 5 $\mu\text{c}/\text{gm}$ body weight. In order to gain further information on the life cycle of lymphocytic cells, it is planned to complement some of the thymidine studies in progress by labeling autologous lymphocytes of a patient with lymphatic leukemia with H³-cytidine, which is incorporated both into DNA and RNA, and to follow their fate after reinfusion into the patient by serial blood and bone marrow sampling. It has been shown by a pilot in vitro study, that about 90% of the lymphocytic cells of this patient are labeled with cytidine.

The amount of H³-cytidine to be used for an in vitro tagging of the autologous cells is 1 $\mu\text{c}/\text{ml}$ of incubation blood. Thus, the maximum H³-cytidine dose to a patient (using 500 ml of blood for cell labeling) would be 500 μc or 0.01 $\mu\text{c}/\text{gm}$ body weight which is one tenth the usual dose of H³TDR. In animals, H³-cytidine and H³-thymidine have about the same toxicity. Dose "estimations" for H³-cytidine would be about the same as for thymidine as discussed in approved H-52.

Dok. G. C. Cotzias

EPC:aw

1179383

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: 30 July 1962

TO: George Cotzias, M. D.

FROM: Eugene P. Cronkite, M. D. 

SUBJECT: Use of tritiated pyrimidines
in patients.

The attached request for the use of tritiated pyrimidines was originally submitted to the isotope committee on 28 February 1961 and approved by all members as indicated on the attached original copies with the exception of Dr. Farr who retained it for over one year before returning it to me. I have rewritten it and up-dated it.

There is now some urgency since we have patients that might be suitable for the administration of tritiated cytidine.

EPC:aw

1179384

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: 30 July 1962

TO: Chairman, Radioisotope Committee
George Cotzias, M. D.
FROM: Eugene P. Cronkite, M. D.

SUBJECT: Use of tritiated pyrimidines in patients in addition to tritiated thymidine. (Refer to isotope authorization H-52.)

1. Tritiated cytidine, deoxyuridine, uridine and other tritiated pyrimidines are precursors for both DNA and RNA. Extensive studies have been performed with these on rats without any detectible evidence of injury at levels up to $1 \mu\text{C}/\text{gm}$ of body weight. With tritiated thymidine, the studies are rather clearcut. It is necessary to give doses in excess of $1 \mu\text{C}/\text{gm}$ to obtain any detectible radiation injury. Between 1 and $5 \mu\text{C}/\text{gm}$ there is negligible effect, with doses of $20 \mu\text{C}/\text{gm}$ in a mouse, the effect is approximately equal to that produced by 50 to 75 rads of external radiation when measured with the testicle and the lymphnodes. The evidence for radiation injury has been measured by the decrease in the number of spermatogonia (Johnson and Cronkite, reprint attached) and by the appearance of pyknotic lymphocytes in the lymph nodes.
2. To date the tritiated pyrimidines with the exception of thymidine have not been administered to man. The problems concerned with the computation of dose are similar to those expressed in H-52.
3. There is some evidence that the leukemic cell may have a metabolic block against the incorporation of thymidine into DNA and that tritiated deoxyuridine or cytidine may label more satisfactorily. These observations are based upon in vitro studies. The evidence for the former is based on studies by Dr. Feinendegen in this Laboratory as yet unpublished. Since this is a key problem in the interpretation of the kinetics of cell proliferation in malignant, it is requested that we be given authority to administer tritiated pyrimidines other than thymidine in doses up to $0.2 \mu\text{C}/\text{gm}$ of body weight. The only patients for which this authority is requested are patients with leukemia, multiple myeloma, other malignant blood dyscrasias or solid cancers that may become available for study. It will be limited to individuals in whom there is no likelihood of procreation and in whom life expectancy is limited. In all of these situations it is noted that radiation therapy is an acceptable and at time a very satisfactory treatment. The amounts of radioactive material given as compared to the amounts of radioactive phosphorus that would be used therapeutically and which also is incorporated into RNA and DNA is very small indeed. Single doses of radioactive phosphorus up to 6 mc are acceptable means of therapy in the leukemic states. The rad

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30 July 1962

dose from this amount of material averaged throughout the body is of the order of 100 rad and in the target tissue may give an integral dose in excess of 200-300 rad depending upon how the dose is computed. As a matter of fact, these dose computations are in reality futile since one does not have enough information on the microscopic distribution of the isotope.

If one considers the pyrimidine activity exclusively as if it were tritium oxide and assuming uniform distribution with a biological half-life of 19 days, the infinite whole-body dose to a 50 kilogram individual is 0.18 rep following the injection of 1 mc. With the present request, it is conceivable that ten times this amount of material would be administered into such an individual resulting in a total body dose of 1.8 rep.

Whereas it is clear that the dangers of tritiated pyrimidines in terms of potential carcinogenic effect and genetic hazards have not been evaluated adequately as yet, it is clear that acute effects from the doses that are needed for adequate autoradiography are not detectible. Acute effects after large doses are minimal to nondetectible. One can compute doses to the bone marrow cells as follows. The volume of bone marrow nuclei assuming spheres will vary from 100 cu. microns (radius about 3 microns to 1300 cu. microns with radius of about 7 microns). The volume of the cells that perpetuate hemopoiesis is not certain but probably varies between 200 and 500 cu. microns. If one uses Hughes estimate of 30 r to a 200 micron nucleus from 60 disintegrations, one can estimate doses to various primitive hemopoietic cells. Grain counts over the larger nuclei on 30 days exposure are as high as 90 after a dose of .1 $\mu\text{c}/\text{gm}$ to patients. If one assumes an autoradiographic efficiency of 1% there are about 300 disintegrations per day. If the volume is the same, the average maximum dose to the nucleus will be of the order of 150 r the first day. Since cell division in these cells occurs every 24 hours or even more rapidly in some cases, the dose is rapidly cut into half for subsequent time periods. In the above computation, dose was estimated on maximum grain count and a low autoradiographic efficiency factor which overestimates the dose. For the first seven days (assuming 7 mitosis) the maximum nuclear dose would be of the order of 300 r. On the basis of mean grain counts it would be of the order of 70 to 100 r during the first week for the primitive progenitor cells as defined by us on cytologic grounds. It is quite evident that the average dose varies with autoradiographic efficiency and nuclear volume of the cell which perpetuates hemopoiesis but which is not identified with certainty. Since neither is known, these numbers have little real meaning. Lastly, the average dose has no real meaning since only the dose to vulnerable structures is of significance in determining the effect. There is no doubt that tritiated thymidine does produce effects but these effects must be determined by empiric studies, not by computation. Apropos of this, there have been published recently, (Nature, vol. 193, pp. 705-706, 1962) data that shows mutations can be induced in the mouse with tritiated thymidine if one uses quite huge doses repeatedly. In these studies huge doses were used 300 μc spread out into 6 fractions over 2 days. This gave uniform labeling of a wide band of spermatozoa and when these sperm were used for fertilization there was a distinct increase in the incidence of

1179386

30 July 1962

of dominant lethal mutations as determined by killing the pregnant females and examining the fetuses early. Thus, it is proved that mutations can be produced by thymidine. In the case of tritiated pyrimidines that go into both RNA and DNA with relatively the same efficiency as thymidine, the preceding maximal dose estimates will also pertain. However, these dose estimates are of the order that would be used therapeutically for blood dyscrasias and from this standpoint are not clinically contraindicated. Another modifying factor is the implication of the Taylor, Wood, Hughes chromosome replication model on dose. Since the chromatids behave with the exception of the perturbation by crossing over as individual units, the labeled chromosomes will be disposed of in successive divisions according to a binomial distribution. With a chromosomal number of 46 as pertains to man and thus after the fifth division the number of cells having no label will progressively increase. (Cronkite et al, Nature, vol. 189, pp. 153-154, January 14, 1961)

It is true that Painter et al have shown inhibition of cell division with doses as low as 0.02 $\mu\text{c}/\text{ml}$ of culture medium. However, these results can not be carried directly to the mammal, in which material is given intravenously, because the availability times are widely different in the two situations.

Admittedly, it is not feasible to give realistic estimates of the doses of radiation to any of the critical target tissues. However, inasmuch as radiation is a recognized therapeutic medium in the diseases discussed and since there is no empiric evidence of tangible injury at the levels considered it is requested that permission be granted to administer up to 0.2 $\mu\text{c}/\text{gm}$ of body weight to individuals with short life expectancy as a result of malignant blood dyscrasias or other malignant disease and in whom procreation is highly unlikely.

EPC:aw

1179387

10
BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: 28 February 1961

TO: Chairman, Radioisotope Committee

FROM: Eugene P. Cronkite, M. D. *EM*

SUBJECT: Use of tritiated pyrimidines in patients in addition to tritiated thymidine. (Refer to isotope authorization H-52.)

1. Tritiated cytidine, deoxyuridine, uridine and other tritiated pyrimidines are precursors for both DNA and RNA. Extensive studies have been performed with these on rats without any detectable evidence of injury at levels up to $1 \mu\text{c}/\text{gm}$. Tritiated thymidine, the studies are rather clear-cut that one has to go to doses in excess of $1 \mu\text{c}/\text{gm}$ to obtain any detectable radiation injury. A dose of approximately $20 \mu\text{c}/\text{gm}$ in a mouse is approximately equal to 50 to 75 rads of external radiation depending upon whether one examines the testicle or the lymphnodes.
2. To date the tritiated pyrimidines with the exception of thymidine have not been administered to any human beings. The problems concerned with the computation of dose are similar to those expressed in H-52.

There is some evidence that the leukemic cell may have a metabolic block against the incorporation of thymidine into DNA and that tritiated deoxyuridine or cytidine will label more satisfactorily. These observations are based upon in vitro studies. Since this is a key problem in the interpretation of the kinetics of cell proliferation in malignant diseases it is requested that we be given authority to administer tritiated pyrimidines other than thymidine in doses up to $.2 \mu\text{c}/\text{gm}$. The only patients for which this authority is requested are leukemics, multiple myeloma or other malignant blood dyscrasias or cancer that may become available for study. It will be limited to individuals in whom there is no possibility of procreation and in whom life expectancy is limited. In all of these situations it is noted that radiation therapy is an acceptable and at times very satisfactory treatment. The amounts of radioactive material given as compared to the amounts of radioactive phosphorus that would be used therapeutically and which also is incorporated into RNA and DNA is very small indeed. Single doses of radioactive phosphorus up to $6 \mu\text{c}$ are acceptable means of therapy in the leukemic states. The rad dose from this amount of material averaged throughout the body is of the order of 100 rad and in the target tissue may give an integral dose in excess of 200 to 300 rad depending upon how it is computed.

1179388

February 28, 1961

If one considers the pyrimidine activity exclusively as if it were tritium oxide and assuming uniform distribution of biological half-life of 19 days the infinitive whole-body dose to a 50 kilo individual is 0.18 rep following the injection of 1 μc . With the present request it is conceivable that 10 times this amount of material would be administered into such an individual resulting in a total body dose of 1.8 rep.

Whereas it is clear that the dangers of tritiated pyrimidines in terms of potential carcinogenic effect and genetic hazards have not been evaluated adequately as yet, it is clear that acute effects from the doses that are needed for adequate autoradiography are negligible. Acute effects after large doses are minimal. One can compute doses to the bone marrow cells as follows. The volume of bone marrow nuclei assuming spheres will vary from 100 cu. microns (radius about 3 microns) to 1300 cu. microns (radius about 7 microns). The volume of the cells that perpetuate hemopoiesis is not certain but probably varies between 200 and 500 cu. microns. If one uses Hughes estimate of 30 r to a 200 micron nucleus from 60 disintegrations one can estimate doses to various primitive hemopoietic cells. Grain counts over the larger nucleus on 30 day exposure are as high as 90 after a dose of .1 $\mu\text{c}/\text{gm}$ to patients. If one assumes an autoradiographic efficiency of 1% there are about 300 disintegrations per day. If the volume is the same, the average maximum dose to the nucleus will be of the order of 150 r the first day. Since cell division in these cells occurs every 24 hours or even more rapidly in some cases, the dose is rapidly cut in half for subsequent time periods. In the above computation, dose was estimated on maximum grain count and a low autoradiographic efficiency factor which overestimates the dose. For the first seven days (assuming 7 mitosis) the maximum nuclear dose would be of the order of 300 r. On the basis of mean grain counts it would be of the order of 70 to 100 r during the first week for the primitive progenitor cells as defined as a common progenitor. It is quite evident that the average dose varies with autoradiographic efficiency and nuclear volume of the cell which perpetuates hemopoiesis. Since neither is known, these numbers have little real meaning. Lastly, the average dose has no real meaning since only the dose to vulnerable structures is a significance in determining the effect. There is no doubt that tritiated thymidine can produce effects, but these effects must be determined by empiric studies, not computation. In the case of tritiated pyrimidines that go into both RNA and DNA with relatively the same efficiency as thymidine the preceding maximal dose estimates will also pertain. However these dose estimates are of the order that would be used therapeutically for blood dyscrasias and from this standpoint are not clinically contraindicated. Another modifying factor is the implication of the Taylor, Wood, Hughes chromosome replication model on dose. Since the chromatids behave with the exception of the perturbation by crossing over as individual units the labeled chromosomes will be disposed of in successive divisions according to a binomial distribution. With a chromosomal number of 46 as pertains to man after the fifth division the number of cells having no label will progressively increase. The attached preprint of a more detailed descriptio

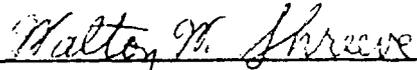
1179389

February 28, 1961

of the effect of the Taylor, Wood, Hughes model on chromosomal replication and the hazard of tritiated thymidine is appended.

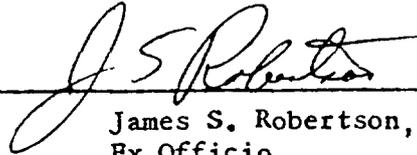
It is true that Painter et al, have shown inhibition of cell division with doses as low as 0.02 $\mu\text{C}/\text{ml}$ of culture medium. However, these results can not be carried directly to the mammal, in which material is given intravenously, because the availability times are widely different in the two situations.

Admittedly it is not feasible to give realistic estimates of the doses of radiation to any of the critical target tissues. However, insomuch as radiation is a recognized therapeutic medium in the diseases discussed and since there is no empiric evidence of tangible injury at the levels considered it is requested that permission be granted to administered up to 0.2 $\mu\text{C}/\text{gm}$ of body weight to individuals with short life expectancy in non-procreative age suffering from malignant blood dyscrasias and other malignant disease.



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

Lee E. Farr, M. D., Ex Officio



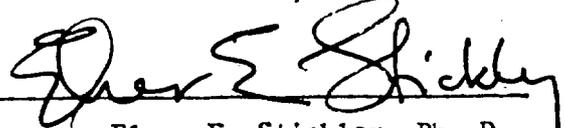
James S. Robertson, M. D. Ph. D.
Ex Officio



Victor P. Bond, M. D., Ph. D.



Samuel Fine, M. D.



Elmer E. Stickley, Ph. D.

EPC:aw

1179390

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: November 14, 1958

TO: Radioisotope Committee

FROM: E. P. Cronkite, M. D. *EM*

PRIVACY ACT MATERIAL REMOVED

SUBJECT: Use of Tritium Thymidine in Patients

1. To date tritiated thymidine's specific activity of 1.9 curies per millimole has been administered in varying dosages to mice and rats. To date with repeated doses of $1/2 \mu\text{c}$ per gram for 7 consecutive days in mice there is no detectable evidence of somatic tissue damage. Up to $800 \mu\text{c}$ has been given to a single mouse without obvious evidence of damage by Dr. Hughes. At a dose of $20 \mu\text{c}$ per gram in a single mouse a moderate amount of diminution in the population of spermatocytes was found in the tubules of the testes 72 hours after the injection of the material. Further studies along these lines are in progress by Dr. Johnson and myself.

2. _____ was given 5 separate injections of tritium thymidine at a level of approximately $1/10 \mu\text{c}$ per gram in 4 of the injections and $2/10 \mu\text{c}$ per gram in one injection. There was no evident change in hematopoiesis following any of these injections. None of the tissues biopsied and none of the tissues at postmortem showed any evidence of obvious somatic effects of radiation. _____ was given one injection of tritiated thymidine at a level of $1/10 \mu\text{c}$ per gram and no harmful effects were detected. He died approximately 4 months after the injection due to his basic disease process and an autopsy was not permitted. The kinetics of hematopoiesis in both _____ and _____ as studied by autoradiography grain counts and appearance and disappearance of labeled cells were identical. One patient with multiple myeloma, two patients with subacute lymphatic leukemia and one patient with subacute myelogenous leukemia have been given tritiated thymidine at $.05$ to $.1 \mu\text{c}$ per gram. No harmful effects have been detected in any of the foregoing individuals. All are still alive and doing well.

3. It is requested that general authority be granted to administer up to $1/10 \mu\text{c}$ per gram of tritiated thymidine to selected patients with fatal blood dyscrasias for both metabolic studies on tritiated thymidine and autoradiographic studies. At the present stage it is appropriate to use the material only in individuals who have acute leukemic process with life span limited to twelve months or less or to individuals in whom their chronic or subacute leukemic process has developed past the age of 50. Under these conditions I am confident that no harm will be done the patient and there is no consideration of genetic damage possible.

EPC:aw

1179391

PRIVACY ACT MATERIAL REMOVED

16 November 1958

Committee on the Use of
Radiosotopes in Humans

from E. E. Stickley

H-52

The attached request for extension of the dosage limits on Project H-52 (Tritiated Thymidine) came through as I was preparing to leave for Chicago. It covers the additional backing which the Committee suggested when the project was initially authorized.

I am circulating copies to the Committee for immediate information. My understanding is that the questions and conditions raised earlier have been satisfied. The Committee will meet approximately 25 November to consider the subject. In the meantime, if there is need for authority, it will be referred to Dr. Farr.

~~Dr. Farr~~
Dr. Robertson
Dr. Borg
Dr. McGinniss
Dr. Shreeve

Attached first copy for master file.

1179392

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: April 2, 1958

TO: File Memorandum

FROM: Committee for the Use
of Isotopes in Humans

SUBJECT: Approval for the Study of Metabolism
and Tissue Uptake of Tritium-Labeled
Thymidine in Human Beings. Project

Approval is granted for the project to study the metabolism and tissue uptake of tritium-labeled thymidine in human beings as presented in the proposal of V. P. Bond, R. A. Conard, E. P. Cronkite, W. L. Hughes, J. R. Rubini, H. Quastler, and J. S. Robertson, dated November 1, 1957. The specific proposal indicates an initial experiment with 5 mc of H^3 thymidine followed by progressively increased amounts until positive historadioautographs are obtained or a maximum of 50 mc is reached. These experiments will be administered under section 5E of the AEC recommendation and requirements relating to patients of limited life expectancy.

D. C. Borg
D. C. Borg, M.D.

Walter W. Shreeve
W. W. Shreeve, M.D.

R. A. Conard
R. A. Conard, M.D.

E. E. Stickley
E. E. Stickley, Ph.D.

J. S. Robertson
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BROOKHAVEN NATIONAL LABORATORY

DATE: 1 November 1957

TO: Committee for the Use of Isotopes
in Human Beings.

FROM: V. P. Bond, R. A. Conard, E. P.
Cronkite, W. L. Hughes, J. R.
Rubin, H. Quastler, J. S. Robertson

SUBJECT: A Study of the Metabolism of
Tritium Labeled Thymidine and Tissue
Uptake in Human Beings.

1. Isotope. Tritium has a physical half life of 12.4 years. It emits soft beta particles with a maximum energy of approximately 18 Kev. and an average energy of 5.7 Kev. No gamma radiation is emitted. Consequently it presents no external radiological hazard to personnel.
2. Compound. Tritium labeled thymidine, as first prepared by Hughes, is now available commercially from Schwarz Laboratories, Inc. in sterile solution with a specific activity of several hundred millicuries per millimole.
3. Radiation Exposure. The very low kinetic energies of the beta particles restrict their effects to a short range around the tritium atom. Robertson and Hughes have calculated that 53% of the energy of tritium is delivered within a radius of 1 micron from the source and 82% within 2 microns. Moreover, the average dose to a sphere of 1 micron radius will be 11 rads/dis-integration whereas the second micron shell will receive only 1 rad. Thymidine is only incorporated into DNA and as such will label chromosomes and nuclei. There are no data on tritiated thymidine in human beings. Hughes et al., have shown in mice that following an intravenous injection of tritiated thymidine the plasma activity of thymidine tritium approaches zero within a few minutes, and there is a concurrent appearance of tritium within cells. This cellular activity appears to be in thymidylic acid. After a few minutes there is a progressive fall in the tissue concentration

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of alcohol - soluble (thymidylic) activity which is accompanied by the formation of tritiated water. Tritium oxide formation reaches a maximum within an hour and accounts for slightly over half of the administered radioactivity. The remaining activity must then be incorporated into a non-metabolizing form - presumably DNA of the cell nuclei of proliferating tissues.

Using autoradiographic techniques in mice nuclear uptake of the labeled thymidine is evident in normoblasts and lymphocytes, 15 minutes after injection. At one hour we have shown that the generative compartment of the gastrointestinal tract is labeled. From the above it is seen that this compound offers a way to tag the nuclei of cells and in particular this compound will be taken up by dividing cells where 11 rads will be delivered per disintegration with a radius of 1 micron from the source of the disintegration. However the meaning of this "average" dose, and the biological significance of this localized dose to such a small sphere is vague. The above suggests that tritium labeled thymidine might well be explored as a potential therapeutic agent in proliferative disorders such as cancer and leukemia and in fact was developed by Hughes with this in mind. Quoting from Siri's book, page 541,

" If artificial radioactivity is ever to prove a vital tool in the cure of malignancy, the only way of accomplishing this end would seem to be the discovery of organic substances that undergo highly selective localization in various types of cancer cells, in contrast to those of normal tissue and that can be labeled with a suitable radioisotope.. Tritium with low penetrating power of its beta particles and (its rapid turnover in the body) is particularly promising because if it can be put in relatively stable positions in localizing compounds, it will expend most of its ionizing power within the concentrating cells. Potentially it is an ideal radiotherapeutic agent."

There is no data on the toxicity of tritium labeled thymidine. There is every reason to be cautious in its use because the labeled DNA will remain in cells for their own specific life. In some instances this will

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be very short -- hours to days depending upon the turnover of the particular cellular system. There is no positive evidence that DNA or its breakdown products are reutilized in new cell production. Even if this were true the dilution in the total pool would be great. The greatest potential danger would seem to lie in its uptake in the gonads. Subsequent fertilization by labeled sperm might constitute a definite genetic and or embryologic hazard. Accordingly, its use in young human beings, who may later procreate, is not justified. In respect to a late somatic danger there is no data and a meaningful analysis would depend upon knowledge of the nature of cell proliferation that is not now available. If cells of the generative compartment of each tissue have a synchronous regular division and maturation the labeled DNA should be promptly diluted out of the generative compartment where carcinogenesis is in theory possible. If instead of synchronous division the process is random and measured interphase is a mean value with a large variance, primitive cells may remain behind with significant levels of labeled DNA that might be a more potent direct cause of cancerous change. On the positive side, no histologic evidence of radiation injury has yet been observed by us in mice injected with 0.7 μ c of labeled thymidine per gram of mouse and observed for ten days after injection. Further studies on the toxicity of H^3 thymidine in animals are underway.

If one considers the thymidine activity as tritium oxide, assuming uniform distribution and a biologic half life of 19 days, the infinity whole body dose to a 50 kilo individual is 0.18 rep following the injection of 1 mc.

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$$\text{Maximal dose rate in reps/day} = 2.3 \times \frac{\mu\text{c}}{\text{Gm}} \times E_{\text{av}}$$

$$6.6 \times 10^{-3} = 2.3 \times \frac{1000}{70,000} \times .006 \times 24$$

$$\text{Integrated Total dose} = \text{dose rate} \times \frac{T_{\text{eff}}}{.693}$$

$$= 6.6 \times 10^{-3} \times \frac{19}{.693} = .18 \text{ rep}$$

During the first week the dose would be approximately 45×10^{-3} rep. for 1 mc

This calculated dose is the maximum hazard to any personnel around patients per millicurie of material used since they could obtain only a small fraction of the expired tritium oxide.

4. Specific Proposal It is proposed to administer H^3 thymidine to patients under the AEC recommendation and requirements, section 5E,

"It is recognized however that special circumstances may arise which indicate the desirability or necessity for the use of long lived radio isotopes in human subjects where prior animal data are not available. Consideration of such proposals shall be limited to patients suffering from diseased conditions of such a nature (life expectancy of one year or less) that there is no reasonable probability of radioactivity employed producing manifest injury."

Initially patients in this category (terminal brain tumors) will be given 5 mc of H^3 thymidine intravenously (i.e. at 1/8 of the body concentration used in studies in mice) and the plasma clearance, urinary and fecal excretion will be measured. Catabolism to body water will also be followed as perhaps the best method of estimating retention. Chemical determination of fixed tritium in appropriate tissue such as leukocyte concentrates will be attempted. Serial blood smears, bone marrow smears, skin biopsies exfoliative cytology and testicular biopsies will be performed for histoautoradiographic studies.

The dose of H^3 thymidine will be progressively increased (up to 50 mc if necessary) by regular increments until positive histoautoradiographs

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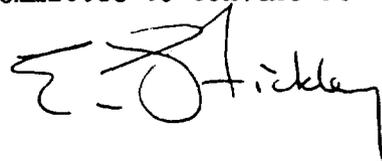
MEMORANDUM

DATE: January 8, 1958

PRIVACY ACT MATERIAL REMOVED

TO: Dr. E. P. Cronkite
FROM: Dr. E. E. Stickley
SUBJECT: Tritiated Thymidine

1. The Isotope Committee grants permission for the tritiated thymidine procedure requested in your memo of this date. The procedure is understood to be the administration of a second dose to , a terminal patient, to the amount of 20 millicuries.
2. Data resulting from the previous procedure is acknowledged and entered as part of the record.
3. Further review of the results of the present experiment is requested. It is understood that the details have been discussed with Dr. Farr and Dr. Robertson and their approval received, in view of the inability of the full committee to convene because of illness.



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are obtained.

When sufficient data has been accumulated on the metabolic behaviour of this material, therapeutic studies involving doses in excess of the maximum doses considered here for metabolic purposes will be considered in selected patients.

5. General Comments. There are many uncertainties in respect to the use of tritiated thymidine. It is reasonable to assume on the basis of the preceding calculations that there is no hazard to personnel from the catabolism of the tritiated thymidine to tritium oxide. There is obviously no external hazard to radiation. The probability of any hazard to personnel are minimal providing ordinary precautions are taken to prevent ingestion of the material. The probable major hazard is a genetic injury. The gonadal dose is 0.18 rep plus the dose from the discrete sources incorporated into DNA. Since the concentration and the microscopic distribution of the H^3 thymidine is not known neither the localized nor its average dose can be calculated. Similar problems exist in respect to all of the proliferative tissues and the possible hazards are discussed earlier.

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BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: January 8, 1958

TO: Isotope Committee

PRIVACY ACT MATERIAL REMOVED

FROM: E. P. Cronkite, M. D. *EPC*

SUBJECT: Tritiated Thymidine

1. Permission is requested to administer another dose of tritiated thymidine to . It is desired to give 20 mc (1.3 curie/milli ml) intravenously on 10 January. The increase in dose is desired in order to shorten exposure time of autoradiography, to get better estimates of the disappearance and degradation of thymidine, and since the higher specific activity will probably improve the uptake it will give a better insight into potential later therapeutic applications. The patient is close to the end now. We estimate life expectancy as a matter of days.

2. To date the following has been observed,

a. Tritium water increased to a maximum approximately 40 minutes after the injection of 9 mc of ³H thymidine. The activity then decreased with a half life estimated at 9 days. Approximately 2 mc of tritiated water was present at 40 minutes.

b. Non-volatile tritium activity has been detected in the urine probably beta amino iso butyric acid. Amounts not yet clear.

c. Other studies are in progress on the metabolic products.

d. Autoradiographs of the bone marrow after 30 days of exposure show slight but definite labeling of the red cell and white cell series. No labeling of megakaryocytes evident.

3. It is regretted that so little time is given for your consideration. However, the probability of having another suitable patient in the near future is remote. Furthermore the present patient is not expected to live for more than a matter of a few days at the most.

EPC:aw

PRIVACY ACT MATERIAL REMOVED

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November 20, 1957

Committee on Use of Isotopes

B. Stickley

H 52 Approval: Tritiated
Thymidine (Cronkite et al.)

1. It is my understanding that the Committee will agree to the proposed use of tritiated thymidine to the amount requested but for use only in agonal or clearly terminal patients.

2. For patients with expectancy estimated in months, further information is required. At present reading 10-day mouse follow-up indicates no ill effect. Six-week studies should be carried out as a guide to later effects. It was considered in passing that 5 mc levels (about 1/8 of the mouse pilot studies) could be attempted for these patients with no harmful effects expected.

3. New and specific instruction for handling patients and wastes on the wards should be issued. All collected wastes should be sealed immediately. We probably should provide positive air removal for the first 24 hours.

4. Gonadal dose estimates are not a stringent requirement in terminal patients. It is noted that a prime purpose of this experiment is the elucidation of this specific point, however, through studies of tissue concentrations taken at appropriate times.

5. It is requested that further information from the continued mouse studies (paragraph 2, above) be added as supplement to the authorization to justify future use in other types of patients.

6. Personnel protection requirements should be revised on the basis of air sampling and other observations by Health Physics surveyors. If preparation of the experimental material is undertaken here, laboratory procedures might also be stipulated from this angle.

7. Paragraph 1 of the Request (draft of November 1, 1957) should acknowledge in an additional sentence that there are hazards from tritium as an internal source, with reference to entry through skin, lungs, or ingestion.

cc: ✓ Dr. Farr
Dr. Robertson
Dr. Stickley
Dr. Rubini
Dr. Borg
Dr. Conard
Dr. Shreeve

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