725549

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: 24 March 1971

TO: A. Harrison

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

SUBJECT: CIRC Numbers

The following CIRC #s are inactive. Except for completing old records, they should not be used for identifying patient activity until reactivation is formally approved.

Principal Investigator	CIRC #
Dr. Cronkite	3
	4
	20
	23
	35
	35A
Dr. Dahl	5
	6
	14
	25
Dr. Jesseph	1
Dr. Robertson	9
	10
	16
	28
Dr. Schiffer	22

The Medical Research Center

Brookhaven National Laboratory

Upton, L. I., New York

EPC/ck
cc: CIRC Committee
Mr. Finn
Dr. Dahl

REPOSITORY Records Holding Area Bldg. 494
COLLECTION Committee - Clinical Investigations and uses of Radioisotopes
BOX No. 4
FOLDER CIRC #23

1179979

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

Treatment of Leukemia and Lymphoma with Tritiated Thymidine (H^3TdR)

CIRC # 23 has been assigned to this program.

George C. Cotzias

George C. Cotzias, M.D., Chairman

Lewis M. Schiffer, M.D.

Knud ~~N.~~ D. Knudsen, M.D.

Walton W. Shreeve, M.D., Ph.D. (ex officio)

Date: AUG 11 1965

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

1179980

Initial Approval

V.P. Bond, M.D.
Chairman, Med. Dept.

FORM FOR INITIATION OR REVIEW OF CLINICAL

INVESTIGATIVE PROGRAMS

(Submit original only to Department Chairman)

A. Title of the proposal: Treatment of Leukemia and Lymphoma with tritiated thymidine (H^3Tdr)

B. Sponsoring physician(s): Eugene P. Cronkite

C. Responsible investigator(s): V.P. Bond, A.D. Chanana, E.P. Cronkite, M.L. Greenberg, L.M. Schiffer, P.A. Stryckmans

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 sheets

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

Please see attached sheets.

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.):

Please see attached sheets.

1179981

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 ___ No X

Note the project number: _____

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.

[signature]
Sponsoring Physician

Committee on Clinical Investigations and Uses of Radioisotopes

Approval recommended ✓ Date 5/13/65

Disapproval _____ Date _____

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

1179982

6/25/63
mlk

SUPPLEMENTARY FORM FOR RADIOISOTOPE

ADMINISTRATION TO HUMAN BEINGS

A. Radioisotope

1. Species: (Radioisotope or labeled compound, eg. $Na^{24}Cl$ or 1-C^{14}- glucose) ^{3}H-Thymidine (H^3Tdr)

2. Physical characteristics: (Physical half-life; decay scheme (or type, energy and relative frequency of major emissions)
T 1/2 - 12.4 yrs. Beta decay 18 KEV $^3H \longrightarrow He + \beta$

3. Source: (BNL reactor, cyclotron, hot lab.), commercial supplier, etc.)

Commercial supplier.

4. Preparation: (Target material, quantity, special problems) It is produced by a catalytic reduction of 5-hydroxymethyluracil with tritium gas to form thymine-^{3} followed by enzymatic conversion of thymine to thymidine. Labeled thymidine is separated and purified by column chromatography.

5. Specific activity and isotopic purity of administered material: The highest available specific activity (presently 15 c/mM) will be used. It is at least 97% pure (usually more than 99%) chemically and is isotopically pure.

6. Radioassay and calibration procedures: (Include validation to be performed at BNL prior to use) Radioassay and calibration are done by the commercial supplier. A radioautogram of a chromatogram is done.

7. Vehicle and route of administration: The aqueous or ethanol solution obtained from the supplier will be injected intravenously.

8. Procedures for control of sterility and pyrogenicity: (Or note that commercially supplied isotopes are certified as ready for administration to human beings.) It is provided by the supplier in a sterile, pyrogen free solution.

9. Extraneous effects, if pertinent: (Such as pharmacological or toxic actions of the parent compound or vehicle, etc.)
None

B. Radiation Dosage

1. Biological half-life or half-lives, including slow components: Biologic half-life will depend upon the specific cells labeled but should be in the order of a few days for the fraction which enters most cells, a few hours for that which is not incorporated and as long as the life of the patient for those cells which turn over very slowly. 20-65% of the injected*

2. Organ, cellular, or subcellular localization: (Should account for the effects of special drugs or agents on altering the natural distribution of the radioisotope)

a. Critical or "target" organ(s): Rapidly dividing cells, especially bone marrow, tumor mucosal and spermatogenic cells.

b. Gonadal exposure: Exposure cannot be measured exactly but should be considerable. Johnson and Cronkite[2] noticed a reduction in the ratio of spermatocytes/Sertoli cells in the mouse testis after giving as little as 1μc/gm intraperitoneally. This effect was the same as 5 rads whole body gamma irradiation.

*H^3Tdr becomes tritiated water with a biologic half-life of 7-12 days (Ref. 1).

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3. Sample calculations: (Dosage should be calculated for the whole body and for "target" or other separate organs, where indicated)
Summary equations are desired; not extensive calculations. Standard dosage equations from references such as Hine and Brownell's Radiation Dosimetry, National Bureau of Standards Handbook 69, and BNL Hospital Form 1167-A should be used where possible and the reference cited.
Dosage calculations must be based on the dose to the nucleus of the proliferating cells which incorporate the H^3Tdr. Such calculations have been reviewed by Bond and Feinendegen[3]. Dosage estimates have been made for the first patient we studied[4].

C. Radiological Health Aspects

1. Hazards to other patients and to personnel from external or internal radiation: There is no hazard to patients or personnel if excreta, etc. are properly handled.

2. Monitoring procedures, if necessary: The Health Physics Department will periodically check the patients room and other areas in the pavillion and ECI room for tritium.

3. Special procedures for handling waste products, excreta, biological samples, etc., where indicated: The patient will stay in his room for 24 hours with his door closed. Urine will be collected completely and disposed as radiation material. Any blood samples drawn the day of injection will be treated as radioactive. Linen, dishes and other materials brought into the room will be treated as radioactive for one day after injection.

4. Plan for isotope accountability, if required: Not required.

Initial Approval:

VB

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

FORM FOR INITIATION OR REVIEW OF CLINICAL INVESTIGATIVE PROGRAMS

(Submit original only to Department Chairman)

A. Title of the proposal: Evaluation of Tc^{99m}-Fe-Ascorbic Acid Complex for Renal Scanning.

B. Sponsoring physician(s): J.S. Robertson

C. Responsible investigator(s): H. Atkins, L. Schiffer

D. Brief description of the study, including its general goals and purpose, and pertinent information on past studies: (Attach additional sheets if necessary.)

The complex of Tc and Fe with ascorbic acid is rapidly excreted from the body in urine. It has been used on rats and we have used it on a rabbit at this laboratory. Superior renal scans can be performed with this material because of the better resolution and higher count rates possible. It is desirable to evaluate its human use. The material has been used on humans at Argonne Cancer Research Hospital by Harper and at Chicago Wesly Hospital by Quinn.

The plan is to administer 1 mc intravenously and follow uptake curves over both kidneys for 30 min, and do fractional urines to determine rate of excretion. Renal scan to be performed at 1 hour. Serial plasma concentration determinations will be done.

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

Material to be used for clinical studies of renal function and anatomical abnormalities.

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.): Patients with hypertension and suspected ureteral obstruction. Will also be used on patients with known abnormal renal function and renal transplants.

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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 X No ___

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): Tc-Fe-ascorbic acid complex.

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 ___ No X

Note the project number: _____

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.

J. B. Robertson
Sponsoring Physician

Committee on Clinical Investigations and Uses of Radioisotopes

Approval recommended ✓ Date 10/24/66

Disapproval _____ Date _____

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

117998b

6/25/63
mlk

SUPPLEMENTARY FORM FOR RADIOISOTOPE
ADMINISTRATION TO HUMAN BEINGS

A. Radioisotope

1. Species: (Radioisotope or labeled compound, eg. $Na^{24}Cl$ or 1-C^{14}- glucose)
   ^{99m}Tc-Fe-ascorbic acid complex
2. Physical characteristics: (Physical half-life; decay scheme (or type, energy and relative frequency of major emissions)
   $T_{1/2}$ = 6 hours, γ = 140 Kev 9.5% internal conversion
3. Source: (BNL reactor, cyclotron, hot lab.), commercial supplier, etc.)
   Hot lab
4. Preparation: (Target material, quantity, special problems)
   See enclosed
5. Specific activity and isotopic purity of administered material:
   Spec. act. = 100% Less than 0.01% impurities Carrier free
6. Radioassay and calibration procedures: (Include validation to be performed at BNL prior to use) Assayed at hot lab by ionization chamber and 57 Co standard
7. Vehicle and route of administration:
   Administered in 0.9% NaCl I.V.
8. Procedures for control of sterility and pyrogenicity: (Or note that commercially supplied isotopes are certified as ready for administration to human beings.) Pyrogen-free sterile solutions used in elution from ^{99}Mo generator. Final product passed through 0.22 μ millipore filter.
9. Extraneous effects, if pertinent: (Such as pharmacological or toxic actions of the parent compound or vehicle, etc.) None. Less than 100 μgm Fe/cc

B. Radiation Dosage

1. Biological half-life or half-lives, including slow components:
   Biological half-life less than 2 hrs in blood. Rapid renal excretion.
2. Organ, cellular, or subcellular localization: (Should account for the effects of special drugs or agents on altering the natural distribution of the radioisotope)
   a. Critical or "target" organ(s): Bladder
   b. Gonadal exposure: .06 rads/m Ci in females assuming full bladder maintained for more than 12 hours. However, this can be considerably reduced by having patient void 1/2 hr post-administration as a routine and then again after completion of scan.
   This is an overestimate of dose and considers all of isotope to be in bladder until physical decay. However, excretion is not that rapid or complete.

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3. Sample calculations: (Dosage should be calculated for the whole body and for "target" or other separate organs, where indicated) Summary equations are desired; not extensive calculations. Standard dosage equations from references such as Hine and Brownell's Radiation Dosimetry, National Bureau of Standards Handbook 69, and BNL Hospital Form 1167-A should be used where possible and the reference cited.

Bladder dose $D_\gamma = C.T\,\bar{g} \times 0.0346 = 0.72 \times \frac{1000\ \mu c}{250\ gm} \times 0.25 \times 0.0346 \times 56$

$\cancel{= 0.72}$

$\bar{g} = 56$ (by interpolation) $= 0.72 \times 0.0346 \times 56$ Values taken from Smith

$C = 1$ mc/250 cc in bladder $= 1.39$ rads Formula from Quimby

Gonad dose - inverse sq law at 5 cm

$$\frac{1.39}{25} = 0.06 \text{ rads/m Ci}$$

C. Radiological Health Aspects

1. Hazards to other patients and to personnel from external or internal radiation: -

2. Monitoring procedures, if necessary: -

3. Special procedures for handling waste products, excreta, biological samples, etc., where indicated: -

4. Plan for isotope accountability, if required: -

Ref: 1. Quimby, E. H. and Feitelberg, S. Radioactive Isotopes in Medicine and Biology. Lea and Febiger, Philadelphia 1963, pp. 118.

2. Smith, E. M. Internal dose calcuation for ^{99m}Tc. J. Nucl. Med. 6: 231-251, 1965.

3. S. Silver. Uses of radioisotopes in medicine. Nucleonics 23: 108, 1965.

ak 1179988 June 25, 1963

D, E, F, B-1, G-4, G-6:

Though the ability of H^3Tdr to kill radiosensitive cells[5] was noted soon after its preparation it came into wide use as a tool to study cellular proliferation because of the high resolution autoradiograms which could be made[6]. No toxicity was seen in animals or man with the tracer doses (up to 0.4μc/gm) Sp. act. 0.3 c/mM to 1.9 C/mM) injected, however[6].

Recently cytocidal effects were observed during studies of cell proliferation in two leukemic patients in this hospital[4,7]. Both of these patients had received much more than the usual tracer dose, each receiving 10 intravenous injections of H^3Tdr over a five day period. Each injection was 0.25 μc/gm body weight. SpA was either 1.9 or 13 c/mM.

A copy of reference 4 is included with this proposal. The data included therein and discussion thereof need not be repeated. A discussion of the toxicity of H^3Tdr is also included.

In addition to studies which will be done with a transplantable mouse leukemia (to be kindly supplied to us by Dr. J.R. Rubini) and further studies on the toxicity of H^3Tdr in the normal dog we feel that the initial observations warrant further therapeutic trials in patients with leukemia (except for chronic lymphatic leukemia and childhood lymphoblastic leukemia) and nonlocalized lymphomas. Because of (1) studies indicating that a large fraction of stem cells is not in DNA synthesis normally[8], (2) the marked granulocytopenia produced by twice daily injections in both our patients[4], and (3) suggestions from clinical trials of other chemotherapeutic agents in acute leukemia that intermittent therapy is superior to daily therapy in maintaining remissions[9], it is proposed to give intermittent rather than twice daily injections of H^3Tdr as we previously did. Because cytotoxic effects depend upon the radiation dose the highest available specific activity material will be used, up to 5 μc/gm body weight per injection. The spacing of injections must necessarily depend upon individual clinical response. An informed consent will be obtained from all patients, of course.

Because of the possibility of inducing mutations in offspring as noted in mice[10], the patients will be informed of the inadvisability of further procreation. The possibility of tumor induction as noted in mice[11], though real, would seem of little importance in view of the generally poor prognosis, even with treatment, in these diseases. Mice given three weekly injections of 10 μc/gm body weight (SpA 1.9 c/mM) did not develop thymic lymphomas, though those given 480r whole body ^{60}Co[12] did. No acute toxic effects have been seen in mice in doses up to 20 μc/gm body weight[2].

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1. J R. Rubini, E.P. Cronkite, V.P. Bond, and T.M. Fliedner, JCI 39:909, 1960.
2. Johnson, H.A. and Cronkite, E.P., Rad. Res. 11:825, 1959.
3. Bond, V.P. and Feinendegen, L.E. Presented at the IAEA panel on Biophysical Aspects of Radiation Quality, held in Vienna, March 29-April 2, 1965.
4. Greenberg, M.L., Chanana, A.D., Cronkite, E.P., Schiffer, L.M., and Stryckmans, P.A. to be submitted to Blood.
5. R.B. Painter, R.M. Drew and W.L. Hughes. Science, 127:1244, 1958.
6. Cronkite, E.P., V.P. Bond, T.M. Fliedner, S.A. Killmann and J.R. Rubini. Presented at the IAEA Symposium on the Detection and Use of Tritium in the Physical and Biological Sciences, Vienna, May 3-10, 1961.
7. M.L. Greenberg, A.D. Chanana, E.P. Cronkite, L.M. Schiffer and P.A. Stryckmans. Clin. Res. 13:274, 1965.
8. T.M. Fliedner, E.D. Thomas, L.M. Meyer, E.P. Cronkite. Ann. N.Y. Acad. Sci, 114:510, 1964.
9. Unpublished observations of the Acute Leukemic B Group.
10. A.J. Bateman, Nature 192:705, 1962.
11. R. Baserga, H. Lisco and W.E. Kisieleski, Proc. Soc. Exp. Biol. and Med. 110:687, 1962.
12. H. Cottier, E.P. Cronkite, E.A. Tonna and N.O. Nielsen. Symposium on Cellular Basis and Aetiology of Late Somatic Effects of Ionizing Radiation, London, 27-30 March, 1962.

1179990

Brief Note

BNL 9374

TRITIATED THYMIDINE AS A CYTOCIDAL AGENT IN HUMAN LEUKEMIA.*

Michael L. Greenberg, Arjun D. Chanana, Eugene P. Cronkite

Lewis M. Schiffer and Pierre A. Stryckmans

Medical Research Center

Brookhaven National Laboratory

Upton, New York

and

The Mount Sinai Hospital

Department of Hematology

New York, New York

Brief Note to Blood, Journal of Hematology

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* Research supported by the U. S. Atomic Energy Commission.
Supported in part by Public Health Service Training Grant #5T4CA-5126-03.

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Running head. Tritiated thymidine cytotoxicity

Send proofs to:

Michael L. Greenberg, M. D.

Medical Research Center

Brookhaven National Laboratory

Upton, New York 11973

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INTRODUCTION:

Though cytocidal effects on radiosensitive cells were recognized[1] soon after the preparation of tritiated thymidine (H^3Tdr),[2] toxicity has not been noted in man in the doses used. During studies with repetitive injections of H^3Tdr in two patients with acute leukemia, however, we obtained evidence of a probable radiation cytocidal effect.

CASE REPORTS:

Case 1. A 74 year old woman entered the Medical Research Center, September 24, 1964, because of progressively severe weakness and pallor for two months and nocturnal fever for several days. She had had diffuse, red skin nodules for one year. Significant physical findings were a temperature of 38.5°C, pallor, scattered 0.5 cm red skin nodules, coarse rales and expiratory wheezes at the right posterior lung base, and a spleen palpable 7 cm. below the left costal margin. On admission the hemoglobin was 6.1 gm %, platelets 18,500/mm^3, and WBC 43,200/mm^3 with 95 % blasts, 3% lymphocytes and 2% segmented neutrophils. A chest x-ray showed a right lower lobe infiltration. A biopsied skin nodule was infiltrated by leukemic cells. A bone marrow aspirate appeared hypercellular with over 90% lymphoblasts. Fever and pneumonia cleared after 10 days of antibiotic therapy. On the fourth day intermittent intravenous H^3Tdr injections (see Materials and Methods) were begun for a study of cell proliferation. Two weeks later the skin infiltrations disappeared and in another week the spleen was only 2 cm below the left costal margin. A bone marrow aspirate was unchanged. One month after the injections the spleen enlarged again; two weeks later she developed a right upper

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lobe pneumonia and died without responding to multiple antibiotics. Transfusions had been given as necessary. Postmortem examination showed a right upper lobe lobar pneumonia and replacement of normal bone marrow and splenic architecture with a uniform population of young lymphocytes.

Case 2. A 42 year old man who received the equivalent of 55 rads whole body 80 Kv x-ray in a criticality accident at Los Alamos[3] was found to have acute myelocytic leukemia in July 1964, after having had three months of increasing fatigue, headaches, and low grade fever. 6-Mercaptopurine, methotrexate, prednisone, and vincristine given at various times prior to his admission to the Medical Research Center on March 30, 1965, had each produced transitory improvement in blood counts as well as various toxic manifestations. On admission, significant physical findings were pallor, 1/2 cm. cervical lymph nodes, a liver 5 cm. and a spleen 1/2 cm. below the lower costal margins, absent deep tendon reflexes and a mild right foot drop. Initially, the hemoglobin was 7.3 gm%, platelets 26,500/mm^3, and WBC 51,200/mm^3 with 4% segmented neutrophils, 8% bands, 6% metamyelocytes, 1% myelocytes, 6% promyelocytes, 10% myeloblasts, 30% monocytoid primitive cells, and 33% atypical primitive mononuclear cells.

Shortly after admission, he developed a meningeal leukemic infiltration. Daily extracorporeal irradiation of the blood,[4] begun immediately and continuted to the present with brief exceptions, decreased the peripheral WBC but had no effect on the infiltration. Intrathecal methotrexate on April 9 and April 20 followed by intramuscular injections of citrovorum factor over the following 24 hours was effective. Splenomegaly increased to 11 cm. below the costal margin. Multiple scattered subcutaneous nodules

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developed; biopsy showed leukemic infiltration. June 15-June 19 he received intravenous injections of H^3TDR (first five with SpA 13 c/mM, second five 1.9c/mM) every 12 hours, each injection being 0.25 μc/gm. The peripheral WBC fell markedly. The spleen shrank to the lower costal margin; the subcutaneous nodules also shrank. A bone marrow aspirate was the same as on admission. On June 20, meningeal infiltration recurred and was again treated successfully with intrathecal methotrexate. Blood transfusions have been given as required. Blood counts are shown graphically (Figure 3).

MATERIALS AND METHODS IN CASE 1:

The patient received ten intravenous injections of H^3Tdr (Sp.Ac. 1.9 c/mM), each 0.25 μc/gm body weight, over a five day period. Autoradiographs of bone marrow aspirates and peripheral blood leukocytes were prepared with Kodak NTB liquid emulsion and developed after 17 days. 1000-2000 blasts were counted on each slide and background corrections calculated on the basis of slides made prior to H^3Tdr injections. The blasts were arbitrarily divided into small (diameter 9-13μ), medium (14-22μ), and large (23-50μ), sizes.

RESULTS OF AUTORADIOGRAPHS IN CASE 1:

Large and medium sized blasts, but not small blasts, were proliferating cells with large fractions initially labeled. The percent of highly labeled large (see Figure 1) and medium sized blasts fell after the fourth injection of H^3Tdr. Though bone marrow interphase cells had up to 80 grains, no mitoses were seen with more than 30 grains. Bone marrow cellularity, judged on the smears of the aspirates, did not change. Changes in total peripheral WBC (differential did not change) are shown in Figure 2. Calculations of certain phases of generative and migration cycles in this patient were previously reported.[5]

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DISCUSSION:

Cytotoxic effects of H^3Tdr have been found in many forms of life. These effects in bacteria and mammalian cells in culture can not be compared with man because of the different concentrations of and time of exposure to H^3Tdr in the medium, but such studies have revealed that the lethal action is due to ionization.[6,7] Rupture of the N-ribosyl bond with resultant loss of the pyrimidine base has been suggested as an effect of ionization.[8] Chromosomes show single breaks and chromatid exchanges after proliferation in H^3Tdr, tritiated water and following exposure to ^{60}Co gamma rays.[9]

Injected H^3Tdr has produced cytotoxic effects in intact mammals. Mouse[10,11] and rat[12] spermatocyte numbers were reduced, as little as 1 μc/gm in a single injection being effective;[10] the proportion of injected H^3Tdr retained was greater when it was given in divided doses than as a single dose.[11] In the rat 25 μc/gm produced as much lymphocyte pyknosis as 75 rads whole body x-ray; whereas 0.5 μc/gm every 12 hours for 17.5 days showed no effect.[13] Up to 1 μc/gm in the mouse, guinea pig, dog or rat or 0.4 μc/gm in man had no apparent effect.[14] Early polyploidy was seen in the liver of rats after 2 μc/gm.[12] Deleterious effects by 1 μc/gm were noted on regeneration of the liver in partially hepatectomized rats.[15] As little as 1 μc/gm delayed recovery of sublethally irradiated mice given bone marrow innocula.[16] Thus, in certain tissues with large fractions of cells in DNA synthesis at one time toxic effects have been noted in doses smaller than the total of 2.5 μc/gm given to the patient. H^3Tdr has also greatly reduced the survival of normal hematopoietic and lymphoma colony-forming cells in mice and prolonged the life of animals bearing the transplanted

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tumor cells by doses of 47-285 μc/gm (Sp.Ac. 13-19 c/mM) divided into six injections at four hour intervals,[17] doses far greater than received by this patient.

One can estimate the radiation dose to the nucleus by H^3Tdr in case 1 by using the calculation by Bond and Feinendegen[18] that in a cell with an average nuclear diameter of 8μ approximately seven rads will be delivered in 24 hours if the average activity were 1.2 disintegrations per hour per nucleus. The mean grain count of large bone marrow blasts at one hour was 11.6 after 17 days autoradiographic exposure or 0.028 grains per hour, corresponding to 0.569 disintegrations per hour if one assumes an autoradiographic efficiency of 5%. Minimum generation time of this blast was 21 hours.[5] If the average generation time for convenience was 24 hours and average nuclear diameter was 8μ (not actually measured but a reasonable estimate for these large cells) then the dose during the first generative cycle would be (0.569/1.2) x (7) or 3.3 rads. Each dividing cell would become labeled again every 24 hours for a total of six labelings if it had continued to divide normally and each mitosis would dilute the label by half. The accumulated dose would then be 3.3 rads at the end of one day, 8.3 rads after two days, 14 rads after three days, 20.2 rads after four days, 26.6 rads after five days, and 33.2 rads after six days. The more heavily cells obviously would receive much larger doses than these average figures indicate, and their death presumably explains the partial remission observed. These calculations must overestimate the dose by the fraction of the generative cycle after mitosis and prior to the next point of labeling. It is more safe to state that dose rate for a singly labeled cell would be 3.3/24 or 0.14 rads per hour to the first division, 0.07 rads per hour to the second division, etc,

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and that with repetitive labeling once every generation cycle, the maximum dose rate would converge upon twice the initial dose rate.

If, indeed, a tritium disintegration produces 10-20 ionizations within 0.1μ of path traversed in a chromosome[19] and if 15-20 ionizations within 0.1μ of a chromatin thread are required to produce a chromatid break with a probability of one[20] in these cells also, then the average cell would have such a break every two hours until time of division, every four hours until the next division, etc. The chance of such a break being lethal necessarily depends upon location and reparative mechanisms.

Spontaneous remissions have been noted in acute leukemia, especially upon recovery from a severe infection and/or fever, especially in children, and often preceded by temporary bone marrow hypoplasia with pancytopenic blood counts.[21] Except for bone marrow hypoplasia and her age, this description fits the first patient. The fall in peripheral white count, decrease in spleen size, and disappearance of leukemic infiltrations in both patients could occur in either a spontaneous or radiation induced partial remission. The loss of the highly labeled segment of the proliferating cells in spite of six additional injections of H^3Tdr and the apparent inability of the most highly labeled cells to proceed into mitosis in case 1 strongly argue for radiation effect, however. Whether these observations are broadly applicable or are unique for two unusually radiosensitive leukemias should be further investigated.

SUMMARY:

1. Two patients had partial remissions of acute leukemias after ten intravenous injections of H^3Tdr (Sp.Ac. 1.9 or 13.0 c/mM), each 0.25 μc/gm body weight, given over five days.

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2. Some aspects of cytotoxic effects of H^3Tdr are reviewed.

3. Radiation dose to the nucleus is estimated.

4. Evidence for the observed remissions being due to H^3Tdr is presented.

1179999

Figure 1. Case 1. Percent label of large blasts in the bone marrow.

BNL # 3-1839-65

1180000

1180001

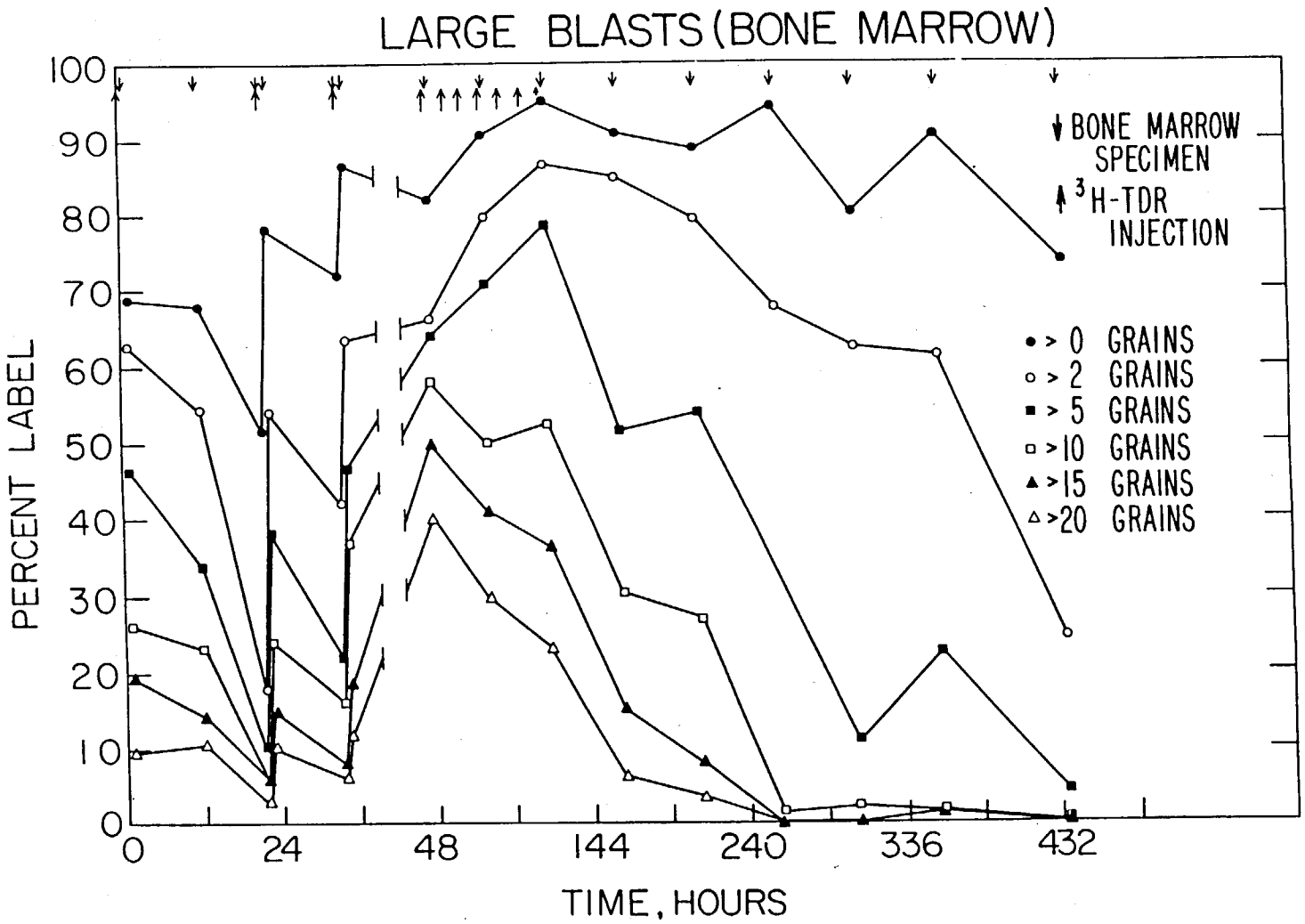

FIGURE 1

Figure 2. Case 1. Total WBC in the peripheral blood.

BNL # 5--60-65

1180002

1180003

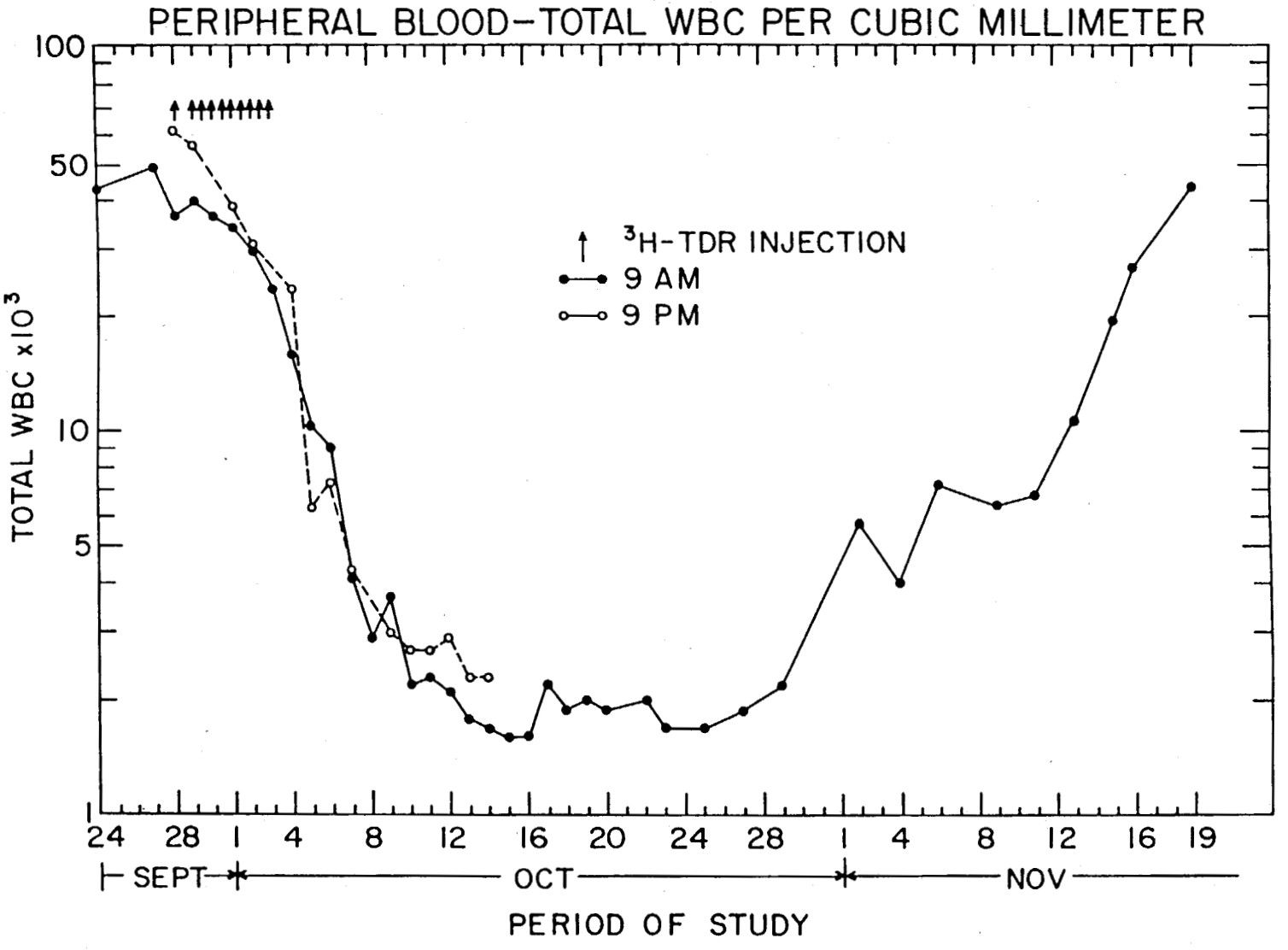

FIGURE 2

Figure 3. Case 2. Peripheral blood counts.

BNL # 7-336-65

1180004

11800005

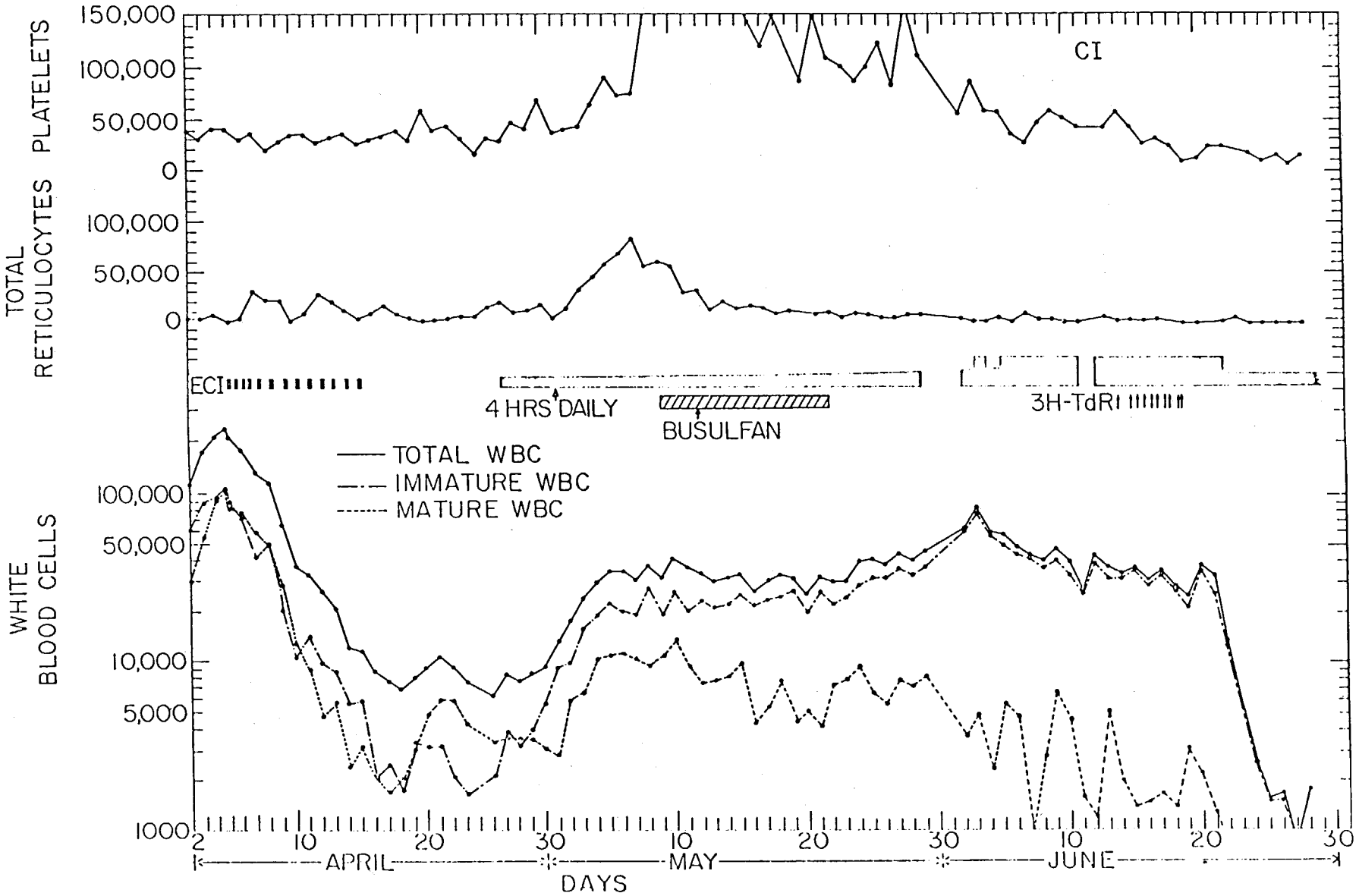

FIGURE 3

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1180008

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1180009

Preparation of Tc-Fe-Ascorbic Acid Complex

The Mo-Tc^{99m} generator is milked with 10 ml of sterile 0.9% NaCl solution. Three ml conc. HCl and 1.0 ml of $FeCl_3 \cdot 6H_2O$ solution containing 8 mg $FeCl_3 \cdot 6H_2O$ or 1.6 mg Fe^{+3} prepared in sterile distilled water, are added and the mixture heated on a water bath for about 10-15 min. One ml of 1% ascorbic acid solution prepared in sterile distilled water is added. The yellow color is faded away ~~by~~ due to the reduction of Fe^{+3} by ascorbic acid. After cooling to room temperature, the pH of the solution is raised to 7.2 by adding NaOH. The solution is kept stirring for about 5 min.

An anion exchange column of Dowex 1 x 8, 50-100 mesh, in the Cl^- form is prepared by putting 2 ml of the resin in a glass column of 10 mm diameter. The resin is washed with about 25 ml of sterile distilled water.

The Tc-Fe-ascorbic acid complex is then run through the column at 0.5 ml/min flow rate. The effluent is then filtered through a sterile 220-micron millipore filter in a sterile sealed vial.

CW:gfs
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