INDIANA UNIVERSITY
SCHOOL OF MEDICINE

NORTHWEST CENTER FOR MEDICAL EDUCATION
3400 Broadway
Gary, Indiana 46408
(219) 980-6550

November 11, 1986

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Remitter
Check No. FEE EXEMPT
Amount
Fee Category 3A 12, 11(a)(4)
Type of Fee
Date Check Rec'd.
Date Completed 11/17/86
By:

Dr. William J. Adams
Materials Licensing Section
U.S. Nuclear Regulatory Commission
Region III
799 Roosevelt Rd.
Glen Ellyn, IL. 60137

Dear Dr. Adams:

We would like to make the following changes in our materials license, number 13-18384-01:

1. Dr. B. Beatrice Chambers is no longer employed by the Northwest Center for Medical Education and we would like to delete her name from our list of responsible investogators.

2. Dr. Roman Dziarski has moved his laboratory to area 1 (see new map of center) and Dr. Hoftiezer has moved his lab into a previously undisignated area now designated Anatomy I laboratory (see new map of the center). Maps of the individual areas are also included as well as a new key to the center map.

3. We would like to add the following responsible investigators to our license: Dr. Thomas Mueller, Dr. Brian Kennedy, and Dr. Subbiah Sivam. Their applications and relevant materials are enclosed. Dr. Mueller will occupy the area vacated by Dr. Chambers (Biochemistry III Laboratory), Dr. Kennedy will occupy a previously undesignated area now designated Physiology IV Laboratory (see new map of the center), and Dr. Sivam will occupy what was formerly designated Microbiology III Laboratory now designated Pharmacology I Laboratory (see new map of the center).

4. Drs. Dziarski and Kennedy have requested that they be licensed for the use of ^{82}Rb and we therefore request that 20 mCi of ^{82}Rb be added to our license. Drs. Dziarski and Kennedy have written protocols for the use of ^{82}Rb which are enclosed.

5. Dr. Kennedy has requested the use of ^{28}Mg in his research and we therefore request that 10 mCi of ^{28}Mg be added to our license. Dr. Kennedy has written a protocol for the use of ^{28}Mg which is enclosed.

6. To accomodate these new responsible investigators and for future expansion we are requesting that our maximum limit of several isotopes be increased. Specifically we request that ^{3}H be increased by 60 mCi (from 80 to 140 mCi); ^{14}C be increased by 10 mCi (from 20 to 30 mCi); ^{32}P be increased by 40 mCi (from 20 to 60 mCi); ^{35}S be increased by 10 mCi (from20 to 30 mCi); ^{22}Na, ^{24}Na, and ^{42}K all be increased by 5 mCi (from 10 to 15 mCi). As stated, these increases are necessitated by increases in our faculty size and for future expansion. When the Northwest Center

originally applied for a materials license, our faculty consisted of six members, and we now have sixteen full-time faculty members at the center.

If you have any questions please contact Dr. Anderson at (219) 980-6556.

Sincerely yours,

W. Marshall Anderson

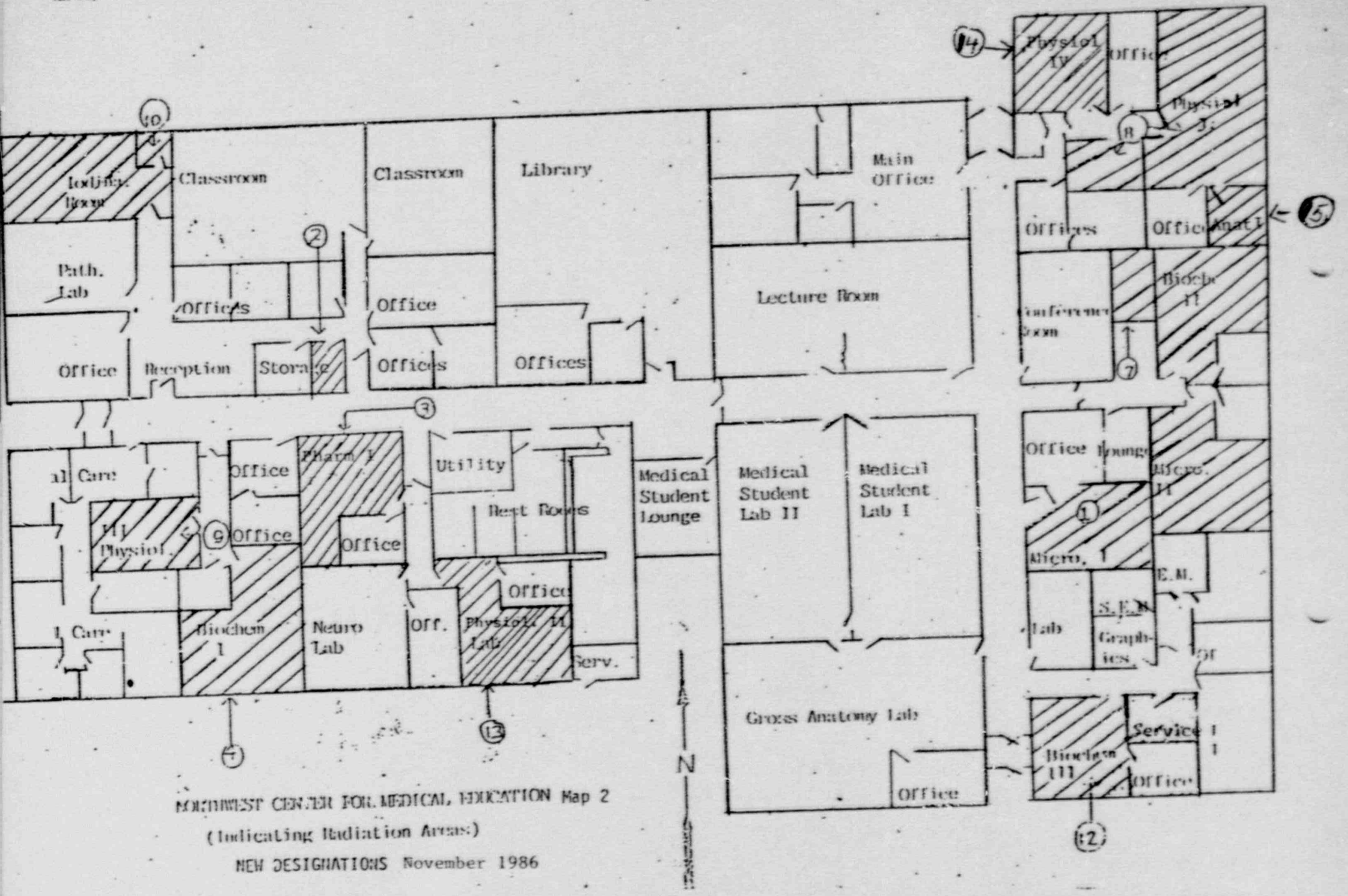
W. Marshall Anderson, Ph.D.
Chairman Radiation Safety Committee

Sincerely yours,

Panayotis G. Iatridis, M.D., D.Sc.
Professor of Physiology and Medicine
Assistant Dean and Director

Enclosures

CONTROL NO. 82462



NORTHWEST CENTER FOR MEDICAL EDUCATION Map 2

(Indicating Radiation Areas)

NEW DESIGNATIONS November 1986

KEY TO NORTHWEST CENTER MAP 2-NEW DESIGNATIONS NOVEMBER 1986

1. Microbiology I Laboratory- Dr. Dziarski
2. Counting Room-General Use Area
3. Pharmacology I Laboratory - Dr. Sivam
4. Biochemistry Laboratory-Dr. Hadd
5. Microbiology II Laboratory-Dr. Baldwin
6. Biochemistry II Laboratory-Dr. Anderson
7. Cold Room-General Use Area
8. Physiology Laboratory-Dr. Iatridis
9. Physiology III Laboratory-Dr. Macchia
10. Radioisotope Storage Room- General Use Area
11. Iodination Room- General Use for Iodination
12. Biochemistry III Laboratory-Dr. Mueller
13. Pyysiology II Laboratory-Dr. Echtenkamp
14. Physiology IV Laboratory-Dr. Kennedy
15. Anatomy I Laboratory-Dr. Hoftiezer

Form 101

APPENDIX H
Application for Approval as a User of Radioactive Material

Name Thomas J. Mueller

Title Assistant Professor

1. Experience

Indicate training in specific areas listed below:

a.

Type of Training	Where Trained	Duration of Training	On the Job	Formal Course
Principles of radiation protection.....	St. Jude Children's Research Hospital Memphis, TN 38101	13 1/2 yrs	(Yes)/No	Yes/(No)
Radioactivity measurement standardization and monitoring techniques and instruments	"	13 1/2 yrs.	(Yes)/No	Yes/(No)
Mathematics and calculations basic to the use and measurement of radioactivity.....	"	13 1/2 yrs	(Yes)/No	Yes/(No)
Biological effects of radiation.....	"	13 1/2 yrs	(Yes)/No	Yes/(No)

b. Experience with Radiation

OTOPE	MAXIMUM AMOUNT	WHERE EXPERIENCE WAS GAINED	DURATION OF EXPERIENCE	TYPE O USE
H	100 mCi	St. Jude Children's Research Hospital	13 1/2 yrs.	Cell and protein labeling
I	15 mCi	"	"	"
I	15 mCi	"	"	"
P	10 mCi	"	"	"
Ca	0.5 mCi	"	"	"

Form 101 Cont.

2. Indicate isotope(s), maximum amount you plan to have at any one time (including waste) and chemical form:

^{125}I as NaI or bound to protein – 10 mCi

^{32}P as inorganic phosphate or ATP – 10 mCi

^{3}H as $NaBH_4$ – 30 mCi

^{14}C as formaldehyde – 0.5 mCi

3. Describe purpose for which radioactive material will be used:
Primarily used for radioactive labeling of cell membranes and proteins/glycoproteins

4. Other persons under your supervision who will be handling radioactive material:

To be named technician

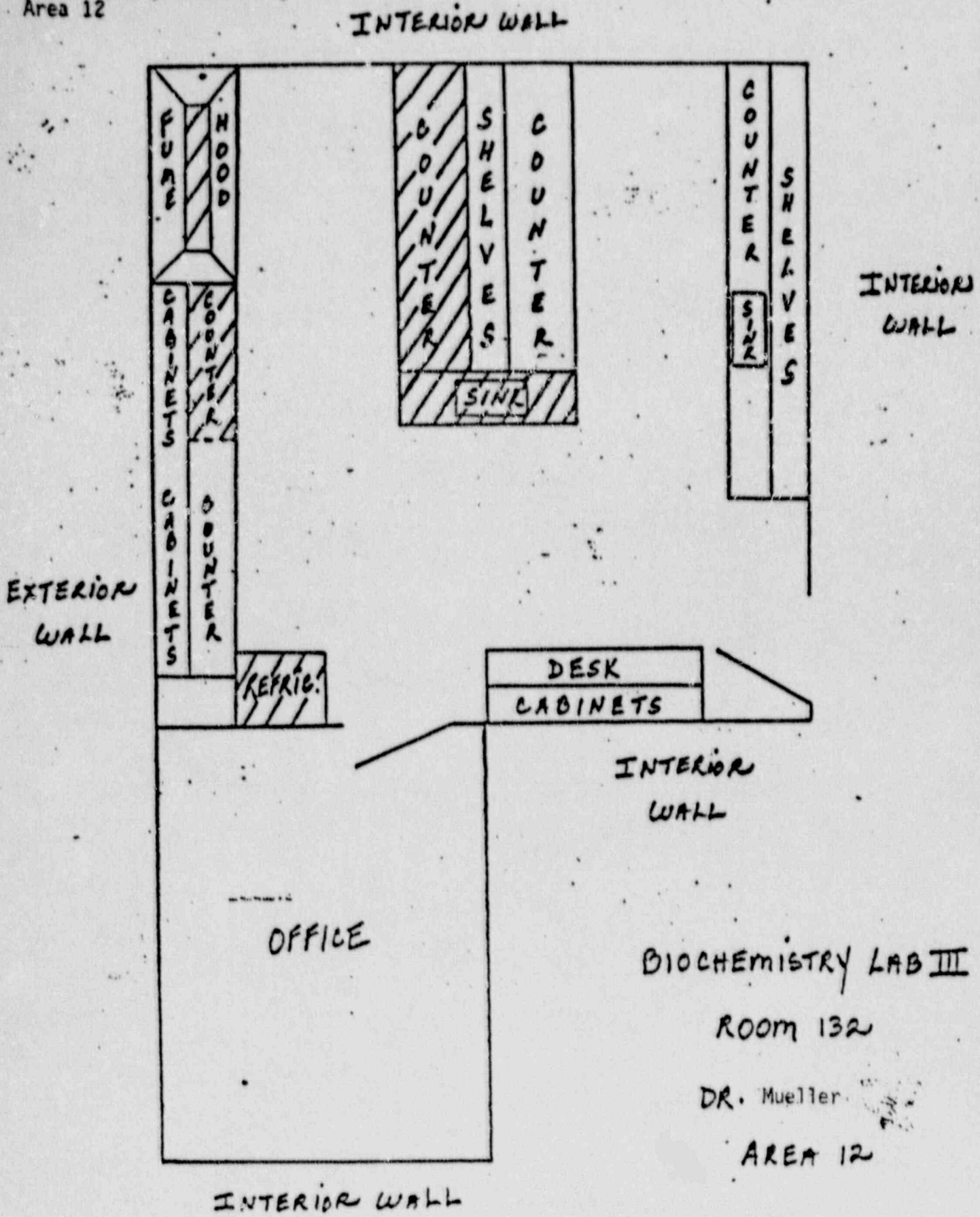
5. Where will radioactive material be used. If appropriate, submit drawing of laboratory, indicating areas of use.
In the hood in my laboratory

6. Describe any special laboratory equipment to be used.

7. Statement of Agreement: The below-named individual signifies that he has read and is willing to abide by the Indiana University regulations governing the use of radioisotopes and other sources of ionizing radiation. The undersigned agrees to comply strictly with all such rules and regulations and further assumes responsibility for ascertaining that employees, students, and associates working under his direction shall comply with the regulations of Indiana University governing the use of radioactive materials and radiation sources.

Date: 7/15/86 Signed: Thomas J. Mueller

Facilities - Dr. Mueller
Biochemistry Laboratory III
Area 12



Form 101

APPENDIX H
Application for Approval as a
User of Radioactive Material

Name Subbiah P. SIVAM

Title Associate Professor of Pharmacology & Toxicology

1. Experience

Indicate training in specific areas listed below:

a. Type of Training	Where Trained	Duration of Training	On the Job	Formal Course
Principles of radiation protection.....	NIH	1 week	(Yes)/No	(Yes)/No
Radioactivity measurement standardization and monitoring techniques and instruments	NIH	1 week	(Yes)/No	(Yes)/No
Mathematics and calculations basic to the use and measurement of radioactivity.....	NIH	1 week	(Yes)/No	(Yes)/No
Biological effects of radiation.....	NIH	1 week	(Yes)/No	(Yes)/No

b. Experience with Radiation

ISOTOPE	MAXIMUM AMOUNT	WHERE EXPERIENCE WAS GAINED	DURATION OF EXPERIENCE	TYPE OF USE
^{32}P	mCi	- NIH	3 years	RIA
^{3}H	mCi	- University of Mississippi - NIH	3½ years 3 years	Receptor binding
^{125}I	mCi	NIH	3 years	RIA
^{14}C	mCi	University of Mississippi	3½ years	Metabolism
^{35}S	mCi	NIH	3½ years	Protein

Form 101 Cont.

2. Indicate isotope(s), maximum amount you plan to have at any one time (including waste) and chemical form:

^{32}P	20 mCi
^{3}H	20 mCi
^{125}I	30 mCi
^{14}C	1 mCi
^{35}S	10 mCi

3. Describe purpose for which radioactive material will be used:

^{125}I will be used for radioimmunoassays; ^{3}H will be used for receptor binding and radioimmunoassays. ^{32}P will be used for molecular biology work eg nick-translation, reaction, end-labeling riBOprobe labelling ^{14}C will be used for pharmacokinetics; ^{35}S is for incorporation studies

4. Other persons under your supervision who will be handling radioactive material:

Technician, Post-doctoral fellow, graduate students (to be named)

5. Where will radioactive material be used. If appropriate, submit drawing of laboratory, indicating areas of use.

Northwest Center for Medical Education Room 122

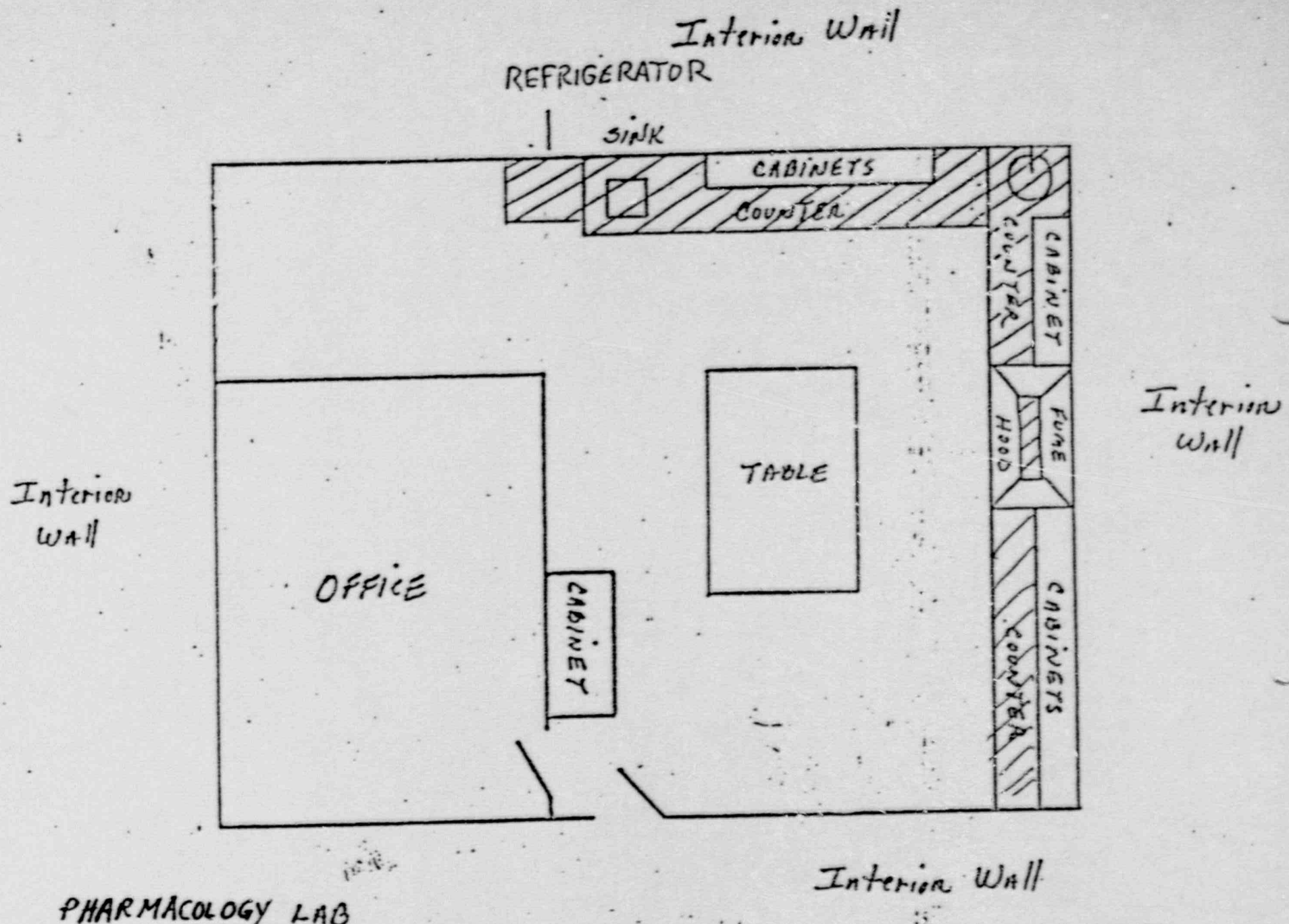
6. Describe any special laboratory equipment to be used.

Radiation shields

7. Statement of Agreement: The below-named individual signifies that he has read and is willing to abide by the Indiana University regulations governing the use of radioisotopes and other sources of ionizing radiation. The undersigned agrees to comply strictly with all such rules and regulations and further assumes responsibility for ascertaining that employees, students, and associates working under his direction shall comply with the regulations of Indiana University governing the use of radioactive materials and radiation sources.

Date: 10/9/85 Signed: [signature]

Pharmacology Lab I



Form 101

APPENDIX H
Application for Approval as a User of Radioactive Material

Name BRIAN KENNEDY

Title ASSISTANT PROFESSOR

1. Experience

Indicate training in specific areas listed below:

a. Type of Training	Where Trained	Duration of Training	On the Job	Formal Course
Principles of radiation protection.....	Washington U. Med Sch St Louis MO Yale U. Med Sch New Haven CT	4 years 4 years	(Yes)/No	(Yes)/No
Radioactivity measurement standardization and monitoring techniques and instruments	SAME as above	SAME as above	(Yes)/No	(Yes)/No
Mathematics and calculations basic to the use and measurement of radioactivity.....	SAME as above	SAME as above	(Yes)/No	(Yes)/No
Biological effects of radiation.....	SAME as above	SAME as above	(Yes)/No	(Yes)/No

b. Experience with Radiation

OTOPE	MAXIMUM AMOUNT	WHERE EXPERIENCE WAS GAINED	DURATION OF EXPERIENCE	TYPE O USE
1.	1 mCi	Washington U Med School ST Louis MO	4 years	
	1 mCi	"	"	
	"	"	"	
a	5 mCi	Yale U Med School New Haven CT	4 years	
a	"	"	"	
c			"	
	1 mCi	"	"	
a	"	"	"	
b	"	U.N.V. of PENNSYLVANIA Med Sch PHIL. PA.	2 years	
g	"	"	"	
	"	"	"	

Form 101 Cont.

2. Indicate isotope(s), maximum amount you plan to have at any one time (including waste) and chemical form:

22-Na, 24-Na, 42-K, 86-Rb, 28-Mg : all as Chloride Salt in aqueous form
36-Cl. : 3 mCi Maximum /Isotope

32-P, 3-H, 14-C : aqueous form
Radioactively Labeled ATP, Sucrose, Innulin

3. Describe purpose for which radioactive material will be used:
Radioactive tracers used to determine unidirectional ion fluxes across Biological membranes. Radioactively labeled ATP will be used to determine Enzymatic Activity. Radioactively Labeled Sucrose And Innulin Used as Extracellular space markers.

4. Other persons under your supervision who will be handling radioactive material:

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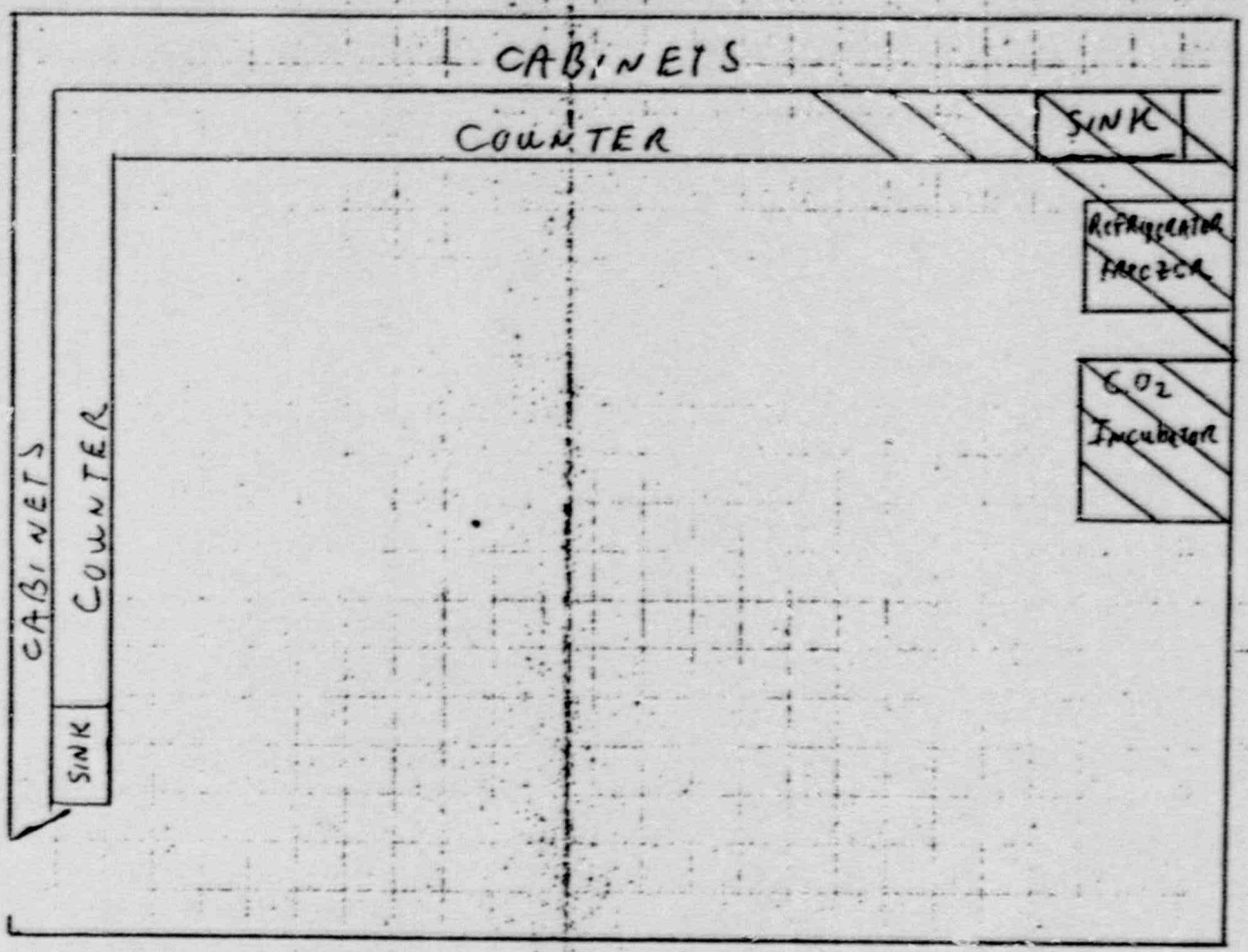
5. Where will radioactive material be used. If appropriate, submit drawing of laboratory, indicating areas of use.

6. Describe any special laboratory equipment to be used.

7. Statement of Agreement: The below-named individual signifies that he has read and is willing to abide by the Indiana University regulations governing the use of radioisotopes and other sources of ionizing radiation. The undersigned agrees to comply strictly with all such rules and regulations and further assumes responsibility for ascertaining that employees, students, and associates working under his direction shall comply with the regulations of Indiana University governing the use of radioactive materials and radiation sources.

Date: 11/3/86 Signed: Brian Kennedy



ROOM 146
Dr. KENNEDY
Physiology LAB III

AREA WHERE ISOTOPES WILL BE STORED / USED

INTER-DEPARTMENTAL COMMUNICATION

NORTHWEST CENTER FOR MEDICAL EDUCATION
AT GARY

DATE November 4, 1986

TO: W.M. Anderson, Ph.D.
Chairman, Radiation Safety Committee

FROM: Roman Dziarski, Ph.D. RD
Associate Professor

DEPT.

DEPT.

SUBJ. Application for the use of ^{86}Kb

TEL. EXT.

I would like to apply for the authorization to use ^{86}Kb, 10 mCi maximum quantity.

^{86}Rb will be used in in vitro studies measuring K^+ uptake by mouse lymphocytes, using the following procedure: lymphocytes will be suspended in the appropriate buffer and mixed with 5 uCi ^{86}Rb per ml; following incubation, 0.2 ml aliquots will be centrifuged through 0.2 ml silicone oil in 0.4 ml polyethylene tubes. The amount of radioactivity in the pellet and the supernatant will be then determined.

cc: P.G Iatridis, M.D., D.Sc., Assistant Dean and Director

Experimental protocol: 28-Mg

Brian Kennedy

Mg^{2+} is, after Na^{+} and K^{+}, the most abundant intracellular cation. It is necessary to maintain cellular integrity and is an essential co-factor for protein synthesis and in a wide variety of enzymatic reactions. Since Mg^{2+} can penetrate cell membranes, and since free intracellular Mg^{2+} activity is held well below electrochemical equilibrium, the cell must possess some energy requiring mechanism capable of net Mg^{2+} extrusion. Very little work has been done to characterize Mg^{2+} transport across biological membranes.

Various metalochromic indicator dyes (such as arsenazo III), intracellular Mg^{2+} specific glass microelectrodes and atomic absorption spectrophotometry can be used to estimate intracellular Mg^{2+} content and to determine net Mg^{2+} transport. To comprehensively characterize the mechanisms mediating transmembrane Mg^{2+} flux, however, unidirectional Mg^{+2} transport must be measured. This can be done in the frog sartorius muscle using 28-Mg as radioactive tracer. Techniques to measure unidirectional cation flux in sartorius muscle are well documented (see Kennedy and DeWeer, for example). Basically, paired, intact muscles are isolated from the frog and suspended in Ringer solution. To measure unidirectional influx, 28-Mg (approx. 1 u A/muscle) is added to initiate the assay. Uptake is allowed to continue for 10-30 min and is terminated by removing the muscle from the radioactive solution. Radioactivity is then extracted from the tissue and counted in a liquid scintellation spectrophotometer. To determine efflux, a muscle is first loaded with 28-Mg by exposure to a 28-Mg labeled Ringer solution for periods up to 48 hours. Efflux is then determined by moving the muscle through a series of test tubes containing unlabeled solution. Radioactivity lost into each tube as well as that remaining in the muscle is counted. Efflux is expressed as a rate constant; i.e., fraction of the total counts lost over a given time period. All labeled solution is saved to allow decay (half life = 21 hours prior to disposal.)

Kennedy, B.G. and DeWeer, P. (1976). J. Gen. Physiol. 68,405.

Experimental protocol: 86-Rb

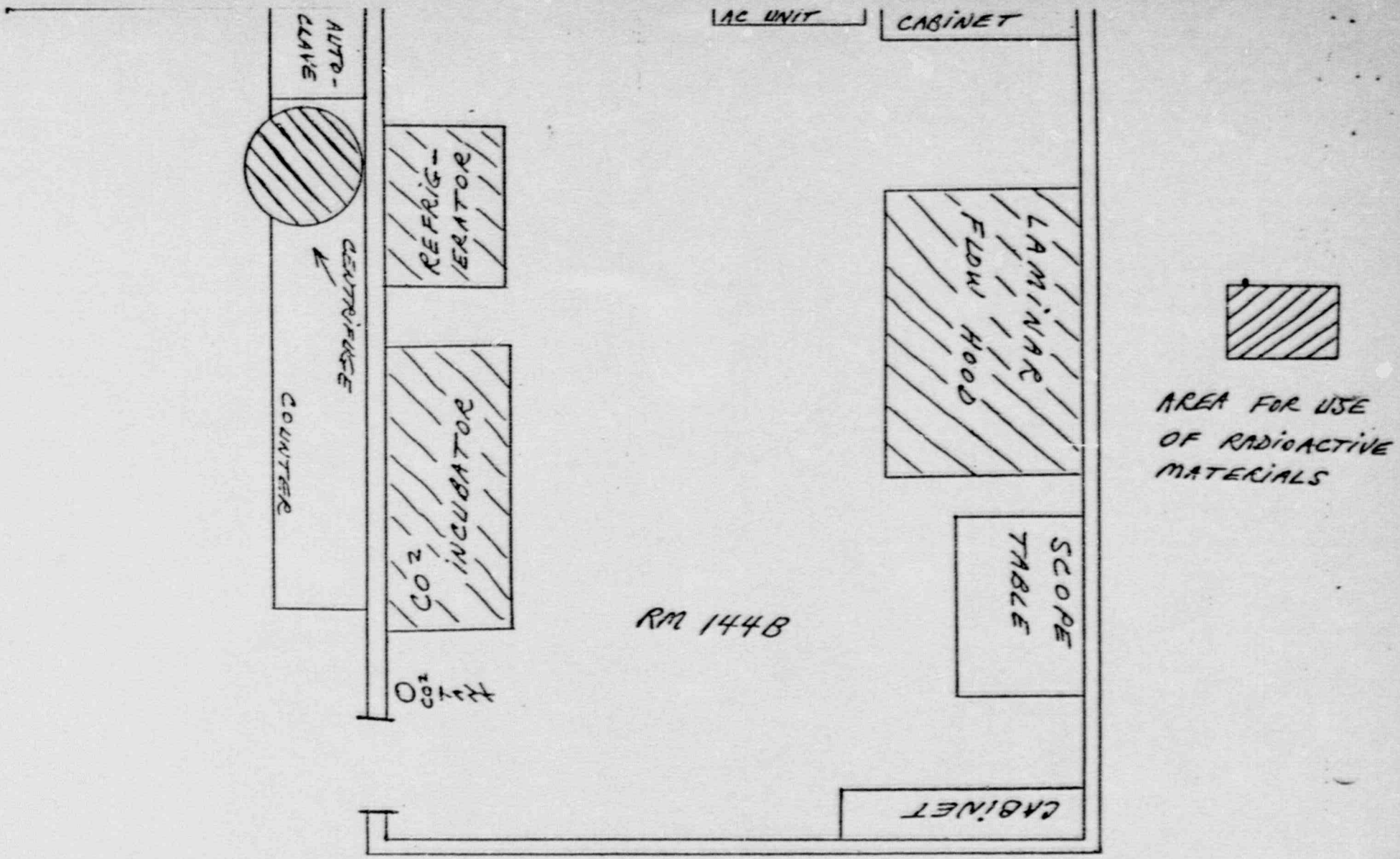
Brian Kennedy

The Na,K-AtPase (commonly referred to as the sodium pump) is a plasma membrane bound enzyme found in virtually all eucaryotic cell membranes. It functions to extrude Na^+ from the cell while accumulating K^+. Energy to drive net ion movement against an electrochemical gradient is provided by the hydrolysis of ATP.

An enormous amount of effort has been expended to elucidate the mechanism of operation of this enzyme (see Forbush and Hoffman for a recent overview). Much information regarding sodium pump function has been gleaned from studies of ion flux across intact cell membranes. Thus K^+ uptake in to a cell is frequently employed as an assay for rate of enzyme turnover. The K^+ isotope, 42-K, is used as tracer to allow measurement of unidirectional K^+ flux. However, 42-K is experimentally rather in convenient due to its short half-life (approx. 12 hours). The external cation binding site of the sodium pump will accept Rb^+ with an affinity comparable to that for K^+ (Robinson and Flashner). Thus 86-Rb is routinely used as a tracer to assay sodium pump function (Kennedy and Lever).

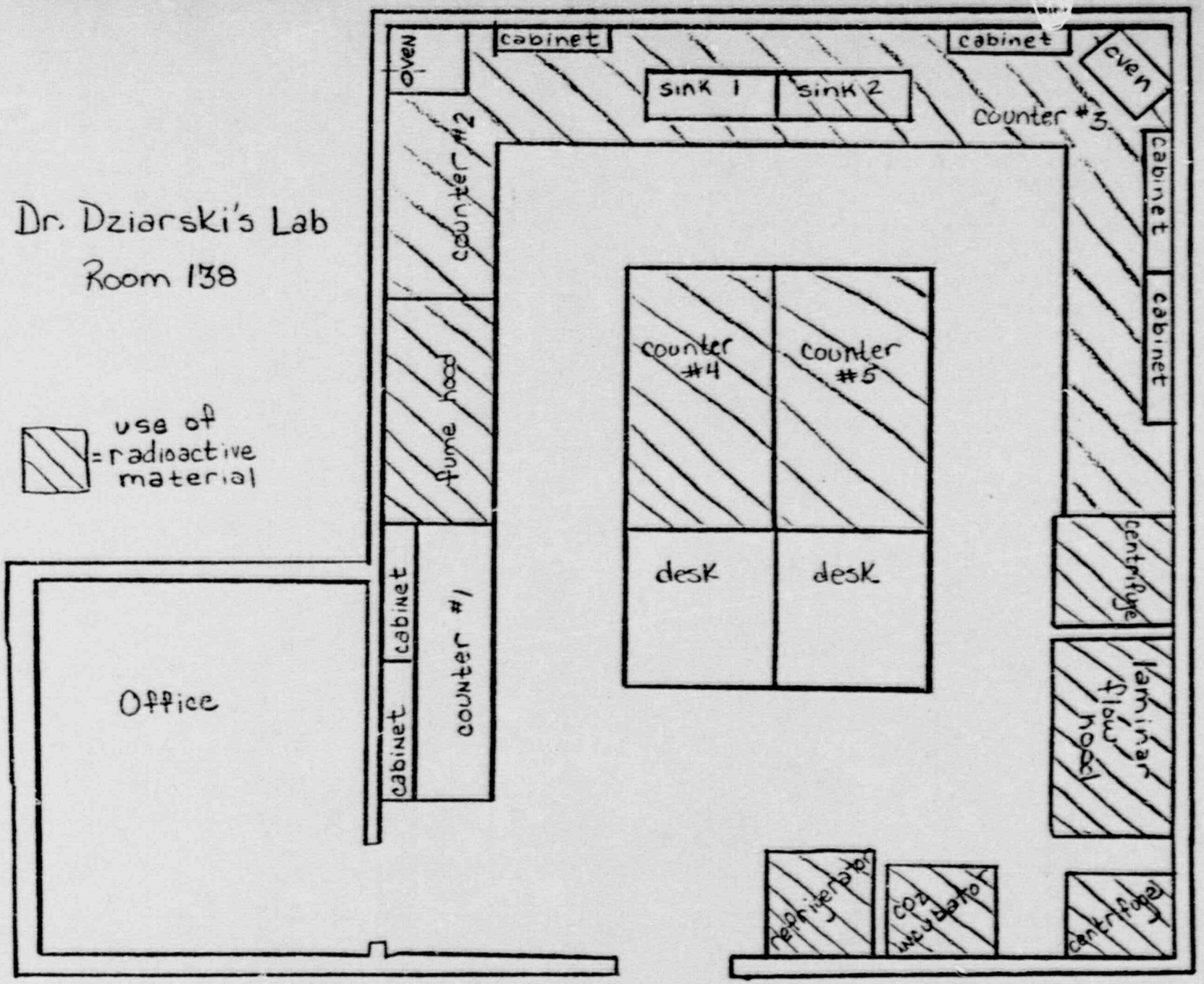
Specifically, I will be examining sodium pump activity in two continuous cell culture lines (the N-16 line, a neuroblastoma cell line and the GH_3 line, of pituitary origin). Both lines can be grown as attached monolayers on 35mm petrie dishes. The cells, as attached monolayers, are exposed to an isotonic saline solution containing 5mMRbCl. The assay is initiated by addition of 86-Rb (approx. 1uCi/dish). After a 5-20 min incubation, the radioactively labeled incubation media is removed. Most of the radioactivity remains in this fraction, which can be stored to allow decay prior to disposal. The cells are solubilized and uptake determined by measuring the amount of radioactivity taken up into the cells. Conveniently, 86-Rb can be cunted in either a gamma counter or in a liquid scintillation counter- with scintillant or with water by cherenkov radiation. Uptake is expressed per mg cell protein, and the cardioactive steroid ouabain is used to define that fraction of Rb^+ uptake actually mediated by the sodium pump.

Forbush, B. and Hoffman, J.F. (1981). Third Int. Conf. on Na, K-ATPase Structure and Function.
Kennedy, B.G. and Lever, J.L. (1984). J. Cell. Physiol. 121,51.
Robinson, J.D. and Flashner, M.S. (1979). Biochem. Biophys. Acta 549,145.



DR. HOFTIEZER'S LAB SPACE
(IN ROOM 144)

SCALE : 1" = 2'


Dr. Dziarski's Lab
Room 138
use of
= radioactive
material
Office
oven
cabinet
sink 1
sink 2
cabinet
oven
counter #3
counter #2
cabinet
cabinet
fume hood
counter
#4
counter
#5
desk
desk
centrifuge
laminar
flow
hood
cabinet
cabinet
counter #1
refrigerator
CO2 incubator
centrifuge