Medical Center
(Atlanta)

1670 Clairmont Road
Decatur GA 30033

Veterans
Administration

Carol Connell
DRSS

In Reply Refer To: 508/115

April 25, 1985

Ms. Carol A. Connell
U. S. Nuclear Regulatory Commission
Region II, Nuclear Materials Safety Section
101 Marietta Street, N.W.
Atlanta, GA 30323

Dear Ms. Connell:

In response to your April 12, 1985 letter (Reference: 18627, 030-01350), the additional information requested in support of our application are as follows:

1. We would like to request authorization to use S-35 for in vitro studies with the maximum possession amount of 100mCi.

2. We would like to increase the possession limit for the H-3 labelled steroid hormone to 5mCi.

3. We would like to obtain a possession limit of 5mCi for C-14 labelled steroid hormone.

4. We request the increase of possession limits of H-3, P-32, and I-125 to the following:

H-2:	250mCi
P-32:	100mCI
I-125:	100mCi

We hope that this information will be helpful for your review.

Sincerely,

GLENN ALRED, JR.
Medical Center Director

Veterans Administration

Memorandum

Date: November 28, 1984

From: Chairman, Radioisotope Committee (115)

To: ACOS for R & D (151)

Subj: Reference to your memo dated 11/9/84 Concerning Authorization for the use of Radionuclides invivo in VAMC

I would like to notify you that you are authorized to administer radioactive hormone(s) on human subjects labeled with ^{14}C and/or ^{3}H as described in your research protocol, with your associates V. Musey, P. Musey, S. Lambert, and D. C. Collins. Until a NRC authorization is obtained, these will be given at Emory Clinical Research Facilities and urine collected would be transferred to the VAMC.

YAVUZ A. TARCAN, M.D.
Chairman, V.A. Radioisotope Committee

Veterans Administration

Memorandum

Date: November 9, 1984

To: Chairman, VAMC Isotope Committee (115)

From: John R.K. Preedy, M.D. (151)
Associate Chief of Staff for Research and Development

Subj: Authorization for the use of radionuclides

1. With reference to your letter, the research protocol under which I would be giving radionuclides to humans were approved provisionally in March, 1983, by Dr. Sankey, previous Radiation Safety Officer, pending presentation to the full Isotope Committee. Apparently, no presentation was made.

2. Since I shall now want to administer radionuclides to humans as part of approved research projects, I ask that the necessary steps be taken to amend my present NRC authorization to include "in vivo in humans".

3. Thank you for your assistance.

File - Radioisotope Comm.
DR. TARCAN

VA FORM 2105
MAY 1983

U.S. GOVERNMENT PRINTING OFFICE: 1984-421-488/0348

EMORY UNIVERSITY -- RADIOISOTOPE COMMITTEE I

APPLICATION FOR HUMAN USE OF RADIOACTIVE MATERIAL

Principal Investigator John R.K. Preedy Department Medicine

Isotope ^{3}H Chemical Form of Isotope ^{3}H-estrogens

Other Persons to use Isotope Victoria C. Musey, M.D.

Possession Limit Requested 1 μCi Lab. No. VA (B-28) Bldg. VA *

Critical Organ None Biological Half-Life 12 hr Weight Critical Organ --

† Microcurie Dose 30-50 % Uptake by Organ -- Secondary Organ -- %

Repeat Study in one Subject? No No. Times -- Material Sterile, Pyrogen Free? Yes

Method of Isotope Administration IV Source of Isotope Supply New England Nuclear or equivalent supplier

Experience of Principal Investigator is (Attached, on file).

Purpose for Study: Indicate the research, diagnostic, or therapeutic value of this study.

EXPERIMENTAL APPROACH: List all steps involving radioisotopes. Attach literature references if available. Use additional pages if necessary.

*Note: Radioactive materials will be administered to the human subjects in the Clinical Research Facility, Emory University. They will be transported from the steroid laboratory in the VA to the Clinical Facility, prior to administration.

†This dose is given with 1-5 μCi ^{14}C (see other sheet).

It is requested that approval be obtained to administer ^{14}C and ^{3}H labelled steroids to human subjects at the Veterans Administration Medical Center, in the research area. The subjects will be females during reproductive life, post-menopausal females and males, according to the attached protocol

J Preedy MD
ACOS Res

con't. on reverse side

Experimental Approach con't.

Faculty Rank _____ Phone No. _____

I CERTIFY that I have become familiar with the EMORY UNIVERSITY RADIATION SAFETY PROCEDURES and will implement the requirements contained therein in the pursuit of this work.

Signature _____ Date _____ Signature _____

Dept. Chairman Principal Investigator

FLOOR PLAN: Show location of storage and use areas. Show building and laboratory numbers.

Experimental Approach con't.

Faculty Rank Professor Nuclear Medicine

Phone No. 329-7181

I CERTIFY that I have become familiar with the EMORY UNIVERSITY RADIATION SAFETY PROCEDURES and will implement the requirements contained therein in the pursuit of this work.

Signature _____ Date _____

Dept. Chairman

Signature _____

Principal Investigator

FLOOR PLAN: Show location of storage and use areas. Show building and laboratory numbers.

ORIGIN OF ESTRIOL IN AGING

J.R.K. Preedy, V. Musey, P. Musey, S. Lambert and D.C. Collins

A. Rationale

1. Statement of Problem

Long-term exposure to endogenous estrogens is thought to be a risk factor for certain diseases, such as breast and endometrial carcinoma. On the other hand in aging, at least in the postmenopausal female, there is reduced exposure to estrogens, and this is thought to account in part for such age-associated conditions as osteoporosis, as well as the less well-characterized features of the postmenopausal state. Thus, it is clearly of the greatest importance to identify and characterize all sources of estrogen exposure.

Certain observations suggest the presence of an unexpected source of estrogen exposure via the estrogen estriol. Previously thought to be a biologially inactive metabolite of the principal active estrogens, estradiol-17β and estrone, estriol is now known to have substantial estrogenic activity in the appropriate assay. Furthermore, there is evidence not only that estriol production is increased in the postmenopausal state, but also that it may be produced by an additional pathway not involving estradiol-17β or estrone.

It thus becomes essential to confirm and characterize this potential additional source of estrogenic stimulation as it relates to aging in the female. It will also be important to determine whether a similar situation occurs in the aging male.

2. Hypothesis and Key Questions

It is proposed that estriol constitutes a substantial and unexpected

source of estrogen activity in the aging (postmenopausal) female and that it is produced by an additional synthetic pathway not involving estrone or estradiol-17β.

3. Specific Objectives

(i) To confirm the previous finding that urinary estriol is increased in the the postmenopausal female relative to estrone and estradiol-17β, as compared with the follicular phase of the menstrual cycle.

(ii) To determine whether the increased urinary estriol varies further with age after the menopause.

(iii) To determine whether groups of postmenopausal women can be identified where estriol is high or low, thus indicating potential high risk groups.

(iv) To determine the source of estriol in the postmenopausal female. To determine specifically whether it comes from an additional biosythetic pathway not involving estrone and estradiol-17β.

(v) To find out whether estriol is derived from plasma dehydroepiandrosterone (DHEA) directly, or via the intermediate found in pregnancy, namely, 16α-OH DHEA. (Neither pathway would involve estrone or estradiol-17β).

B. Work Proposed

Subjects: Volunteers would be obtained from the staff of Emory University and the Veterans Administration, as well as from elsewhere. They would be divided into groups as follows; (n = number in each group).

	Females	Males
Age 20-40	n = 10	n = 10
Age 50-70	n = 10	n = 10

Females age 20-40 would be studied during either the follicular or the luteal phase of the cycle. Particular care would be taken to exclude early pregnancy, and plasma pregnancy tests would be run on the plasma obtained before administration of the radioactivity.

Test Protocols. Two protocols will be used, one with and one without the administration of radioactive steroids.

1. Two 24-hour urines would be collected and two 20-30 ml venous blood samples obtained for analysis of steroid hormones. (In the females age 20-40, the pregnancy test would be carried out at this time).
2. Radioactive hormones would be administered intravenously, and all urine collected for the next 96 hours, or until all radioactivity has been excreted. Radioactive hormones would be administered once only per subject.

The radioactivity administered to each patient would be as follows:

^{14}C-dehydroepiandrosterone (DHEA), 1-5 μCi

and

^{3}H-estrone, 30-50 μCi

(In some cases ^{3}H estriol, 30-50 μCi, will be substituted for ^{3}H-estrone).

Both radioactive steroids would be given together to each subject.

Method of Procurement, Preparation and Administration of Radioactive Steroids

The above radioactive steroids would be obtained from a standard supplier, such as New England Nuclear. The steroids are shipped dissolved in benzene-ethanol. The solvent will be evaporated and the steroid re-dissolved in a convenient volume of pure redistilled 95% ethanol.

Under sterile conditions, using sterile syringes and needles, the appropriate volume (0.05 to 0.15 ml) is transferred to a sterile stoppered tube and diluted to 20-25 ml with sterile normal saline. After mixing by inversion, the solution is taken up in a sterile syringe and injected slowly intravenously under sterile conditions.

The specific activities of the steroids are as follows: ^{14}C-DHEA ca 50 µCi/mmole, ^{3}H-estrone or ^{3}H-estriol, 50 µCi/mmole. Thus, the weight injected would be ca 15/µg DHEA, 160 ng estrogens.

Analyses: The urine would be analyzed for the following radioactive and nonradioactive steroids: estrone, estradiol-17β, estriol, estrone sulfate, estrone glucosiduronate, estradiol-17β-17 glucosiduronate and estriol 16α-glucosiduronate.

Interpretation of Results

(i) If we find increased endogenous urinary estriol (or estriol metabolites) relative to estrone and estradiol in the old age group, this will confirm our hypothesis that estriol is quantitatively important in these groups, and will suggest an origin independent of estrone and estradiol-17β.

(ii) If the specific activity of urinary estriol is lower than that of estrone and estradiol-17β, this will prove that estriol is derived from an additional pathway independent of estrone and estradiol. Such a pathway could be estriol secretion as such, or a synthetic pathway not involving estrone and estradiol-17β.

(iii) If the ^{3}H/^{14}C ratio in urinary estriol is found to be lower than estrone, then this will prove that there is a specific pathway from DHEA to estriol which does not involve estrone (as is the case in pregnancy).

Administration of Radioactive Steroids. These will be given by Dr. Preedy and Dr. V. Musey in the Clinical Research Facility at Emory University. Urine collected would be transfered to the VA Medical Center.