Jerry Holland

UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL

Medical Extension

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FOLDER Training Programs

COURSE IN THE APPLICATIONS OF NUCLEAR PHYSICS TO THE BIOLOGICAL AND MEDICAL SCIENCES

June 30th through July 18th
1947

University of California Medical Center
San Francisco

Director of Course: STACY R. METTIER, M.D., Associate Professor of Medicine; Head of Postgraduate Instruction, Medical Extension

General Chairman: WILLIAM J. KERR., Professor, Chairman, Department of Medicine

Director of Laboratory: JOSEPH G. HAMILTON, M.D., Assistant Professor of Medicine and Radiology

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OFFICERS OF INSTRUCTION

Members of the various faculties,
University of California
and visiting lecturers

ALVAREZ, Luis W., Ph.D., Professor of Physics

AXELROD, Dorothy, M.A., Research Assistant, Radiation Laboratory

CALVIN, Melvin, Ph.D., Associate Professor of Chemistry

CHAIKOFF, E. L., Ph.D., M.D., Professor of Physiology

COHN, WALDO E., Ph.D., Research Chemist, Clinton Laboratory, Knoxville, Tenn.

COPP, D. Harold, M.D., Assistant Professor of Physiology

DOUGHERTY, Ellsworth, Ph.D., M.D., Research Fellow in Medical Physics, and Instructor in Zoology

ELLIOTT, Henry W., Ph.D., Research Fellow in Pharmacology

GREENBERG, David M., Ph.D., Professor Biochemistry

HAMILTON, Joseph G., M.D., Associate Professor and Research

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Associate in Medical Physics, Assistant Professor of Medicine

HEIDELBERGER, Charles, Ph.D., Research Chemist, Radiation Laboratory

KERR, William J., M.D., Professor of Medicine; Chairman, Department of Medicine

LANZ, Henry, A.B., Research Assistant, Radiation Laboratory

LAWRENCE, John H., M.D., Assistant Professor of Medicine, Associate Professor and Research Associate in Medical Physics and Research Associate in the Radiation Laboratory

LOW-BEER, Bertram V.A., M.D., Associate Professor of Radiology

McMILLAN, Edwin, Ph.D., Professor of Physics

METTIER, Stacy R., M.D., Associate Professor of Medicine, Head of Postgraduate Instruction, Medical Extension (University Extension)

OVERSTREET, Roy, Ph.D., Associate Professor of Soil Chemistry, Associate Soil Chemist in the Experiment Station

PACE, Mello, Ph.D., M.D., Research Associate in Medical Physics, Radiation Laboratory

PERLMAN, Isadore, Ph.D., Associate Professor of Chemistry in the Radiation Laboratory

SEABORG, Glenn T., Ph.D., Professor of Chemistry

SCOTT, Kenneth, A.B., Research Associate, Radiation Laboratory

SOLEY, Mayo H., M.D., Associate Professor of Medicine, and Assistant Dean of the Medical School

STONE, Robert S., M.D., Professor of Radiology

TARVER, Harold, Ph.D., Assistant Professor of Biochemistry

THORNTON, Robert L., Ph.D., Professor of Physics in the Radiation Laboratory

TOBIAS, Cornelius, M.D., Ph.D., Instructor in Medical Physics, Radiation Laboratory

TOLBERT, B. M. Ph.D., Research Associate in the Radiation Laboratory

YAKEWICH, Peter B., Ph.D., Research Associate in the Radiation Laboratory

ZIEGLE, Raymond E., Ph.D., Professor of Bio-Physics, University of Chicago

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PROGRAM

Note: All lectures and seminars will be given in Cole Hall, 3rd floor, Medical School Building and laboratory exercises in Clinical Pathological Laboratory, main floor of same building.

THERE WILL BE NO ADMITTANCE TO EITHER THE LECTURES AND SEMINARS OR LABORATORY SESSIONS WITHOUT A REGISTRATION CARD.

The First Week: AN INTRODUCTION TO NUCLEAR PHYSICS

Monday, June 30th

8:30-9:30 a.m.	REGISTRATION OF STUDENTS
9:30-10:00	Introductory Remarks Drs. KERR and METZIER
10:00-12 noon	An Introduction to Atomic and Nuclear Structure, Nature of Radioactivity, and Properties of Alpha, Beta, and Gamma Rays. Dr. HAMILTON
1:00-5:00 p.m.	LABORATORY

Tuesday, July 1st

9:00-11:00 a.m.	Nuclear Reactions and The Characteristics, Properties and Production of Neutrons, Deuterons, and Alpha Particles. Dr. HAMILTON
11:00-12 noon	The Cyclotron Dr. THORNTON
1:00-5:00 p.m.	LABORATORY
8:00-10:00 p.m.	The Acceleration of Particles to Very High Energies Dr. ALVAREZ

Wednesday, July 2nd

9:00-11:00 a.m.	The Principles of Radio-Chemistry and its Applications for the Preparation and Isolation of the Artificial Radio-Elements. Dr. Werlman
11:00-12 noon	The Pile Dr. COHN
1:00-5:00 p.m.	LABORATORY

Thursday, July 3rd

9:00-11:00 a.m.	Health Protection and Safety Dr. STONE
11:00-12 noon	Production of Radio-Elements by the Pile at Oak Ridge Dr. COHN
1:00-5:00 p.m.	LABORATORY

Friday, July 4th

9:00-11:00 a.m.	Production of Radio-Elements by the Pile at Oak Ridge (contd) Dr. COHN
11:00-12 noon	To be announced
1:00-5:00 p.m.	LABORATORY
8:00-10:00 p.m.	To be announced.

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The Second Week: RADIOACTIVE TRACERS IN BIOLOGY AND MEDICINE

Monday, July 7th

9:00-11:00 a.m. The Principles of the Applications of Radioactive Tracers and a Detailed Discussion of the Three Major Tracer Techniques.
Dr. HAMILTON

11:00-12 noon Special Techniques for the Measurement of Radioactive Isotopes of Hydrogen, Carbon and Sulfur.
Dr. YANKWICH

1:00-5:00 p.m. LABORATORY

Tuesday, July 8th

9:00-11:00 a.m. Radio-Iodine and Its Application to the Study of the Physiological Study of Iodine
Dr. HAMILTON

11:00-12 noon Special Techniques for the Measurement of Radioactive Isotopes of Hydrogen, Carbon and Sulfur (continued)
Dr. YANKWICH

1:00-5:00 p.m. LABORATORY

8:00-10:00 p.m. Radio-Chemistry and the New Elements
Dr. SEABORG

Wednesday, July 9th

9:00-11:00 a.m. Radio-Phosphorus and Its Use in the Study of Phosphorus Metabolism. Tracer Studies in Normal Animals and in Leukemia.
Dr. DOUGHERTY

11:00-12 noon Preparation of Labelled Compounds Using Carbon 14
Dr. CALVIN or Dr. HEIDELBERGER

1:00-5:00 p.m. LABORATORY

Thursday, July 10th

9:00-11:00 a.m. Radio-Carbon: A Survey of the Applications of this Radio-Element and the Scope of the Future Potentialities of Its Use.
Dr. CALVIN or Dr. HEIDELBERGER

11:00-12 noon Preparation of Labelled Compounds Using Carbon 14 (continued)
Dr. CALVIN or Dr. HEIDELBERGER

1:00-5:00 p.m. LABORATORY

Friday, July 11th

9:00-10:00 a.m. Radio-Iron as a Tool for Studying the Formation of Haemoglobin and Related Problems
Dr. COPP

10:00-11:00 a.m. Radio-Sulfur: The Labelling of Sulfur Containing Compounds Such as Thiamin and Methionine.
Dr. TARVER

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Friday, July 11th (continued)

11:00-12 noon — Thyroxine and Diiodotyrosine Studies with Radioactive Iodine
Dr. CHAIKOFF

1:00-5:00 p.m. — LABORATORY

8:00-10:00 p.m. — The Chain Reacting Pile
Dr. McMILLAN

The Third Week: **RADIO-ACTIVE TRACERS, THERAPEUTIC AND DIAGNOSTIC APPLICATIONS OF THE RADIO-ELEMENTS AND HEALTH-PHYSICS**

Monday, July 14th

9:00-10:00 a.m. — Representative Examples of Tracer Experiments Using the Isotopes of Hydrogen, Sodium, Potassium, Chlorine, Bromine and Strontium
Dr. HAMILTON

10:00-11:00 a.m. — Nitrogen as Tracers and Inert Gas Exchange
Dr. PACE

11:00-12 noon — Radioactive Tracers in Plant Physiology and Soil Chemistry
Dr. OVERSTREET

1:00-5:00 p.m. — LABORATORY

Tuesday, July 15th

9:00-11:00 a.m. — Use of Heavy Isotopes and Tracer Studies with Oxygen
Dr. PACE

11:00-12 noon — Tracer Studies with Radio-Calcium and Radio-Strontium
Dr. GREENBERG

1:00-5:00 p.m. — LABORATORY

8:00-10:00 p.m. — Topic to be announced
Dr. ZIRKLE

Wednesday, July 16th

9:00-11:00 a.m. — The Use of Radioactive Isotopes in Clinical Diagnoses
Dr. LOW-BEER

11:00-12 noon — Radioactive Tracers in the Study of the Physiological Chemistry of Bone
Dr. COPP

1:00-5:00 p.m. — LABORATORY

Thursday, July 17th

9:00-10:00 a.m. — The Therapy of Hyperthyroidism with Radio-Iodine
Dr. SOLEY

10:00-11:00 a.m. — The Use of Radioactive Phosphorus in Therapy
Dr. LOW-BEER

11:00-12:00 noon — The Biological Action of Ionizing Radiations
Dr. ZIRKLE

1:00-5:00 P.M. — LABORATORY

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Friday, July 18th

9:00-11:00 a.m.	Health Physics and Radiation Protection Dr. STONE
11:00-12 noon	The Biological Action of Ionising Radiations (continued) Dr. ZINKLE
1:00-5:00 p.m.	LABORATORY
8:00-10:00 p.m.	General Discussion and Question Box Chairman: Dr. STONE

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Jerry Holland

OUTLINE OF LABORATORY PROCEDURES
FOR
PHYSICS 126 L

by

J. G. Hamilton and K. G. Scott

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LABORATORY SYLLABUS FOR BIOPHYSICS

The main instruments to be used in these exercises will be the Lauritsen electroscope and a Geiger Muller counter. The electroscope used has a very fine quartz fiber which is gold plated and is attached to an amber post and, in addition, has cemented to it a cross bar which can be viewed through the eye piece. When an electric charge of about 200 volts DC is applied to the electroscope, the quartz fiber is bent away from the stationary post since they have the same charge. This moves the cross bar closer to zero on the scale. The amount of charge or deflection of the cross bar depends upon the voltage applied. Even in the absence of a radioactive sample, the charge will be gradually reduced because of electrical leakage in the system, and the ionization caused by the cosmic ray radiation, as well, . . the radiations from surrounding objects. In an electroscope of average sensitivity, some 10 to 20 minutes might elapse for the cross bar to cover 10 small divisions on the scale. The electroscope is charged from 1 to ½ large division more than the region on the scale through which the measurements are made.

The first maneuver should be to determine the background of the particular instrument used for 5 divisions in the portion of the scale to be used. This may be of the order of .005 division a second. A background should be taken each time the instrument is used. Since the background varies with the measuring instrument and the surrounding conditions and not the sample being run, it is always subtracted from the combined rates of discharge of a radioactive sample and the background itself. For example, if it is found that a radioactive sample plus the background discharges the electroscope at a rate of .05 divisions per second and the background is .005, it can be seen that the activity contributed by the sample alone would be .045 divisions per second. An examination of the electroscope will show that the aluminum can surrounding the fiber has been modified so that a very thin aluminum foil window is placed underneath the sample holder. This permits beta particles to enter the instrument. Great care must be exercised in placing samples near the window since it is very fragile and can be easily punctured. Samples should not be placed in the sample holder of the electroscope which are distinctly warmer or colder than the surrounding temperature since these will cause convection currents in the electroscope. These will cause irratic readings.

Geiger Muller Counter

The Geiger Muller counter is a very simple structure composed of a metallic cylindrical wall and a wire in the center which is tipped with a glass bead. These two parts are enclosed in a bell shaped glass envelope and a very thin mica window is fastened upon the flat part of the bell. The tube is then filled with some monoatomic gas, such as helium, argon, krypton or xenon. In addition, some polyatomic gas is admitted, such as alcohol, in order that the tube will "quench" itself after each count, thereby stopping any further discharge until another ionizing event occurs within the counter. This tube is always connected to some type of circuit which will supply the necessary voltage to allow the tube to operate in the Geiger Muller region (1000 to 2000 volts). In addition to the above, the pulses produced by the tube are amplified by the circuit so that they

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have sufficient magnitude to activate some type of recorder. Most circuits actually send out a signal only after every eighth pulse, or some number following from the geometricl progression of the number 2, such as 16, 32, or 64. These are correspondingly called scale of eight or scale of sixty-four circuits. When a Geiger Muller counter is in operating condition, even though no sample of activity is present, it has a background which is due to (1) cosmic rays and (2) the presence of natural radioactivity in the immediate environment. The background can be reduced by a factor of approximately 2 by shielding the counter tube with one to two inches of lead which cuts down the cosmic ray intensity. Such a shielded tube of the volume used for this course would have a background between one and $\frac{1}{2}$ counts per second.

EXERCISE I

Decay curves.

Standarization of counter and electroscope.

Cross calibration of counter and electroscope.

If the intensity of a radioactive material is known for some time period, it is possible to calculate the decay for other time periods through the use of a formula such as:

$$I_t = \frac{Io}{2^{\frac{\text{Time elapsed}}{\text{Half life}}}}$$

Example 1.

Activity I_o = 10 microcuries

Half life = 5 days

Time elapsed = 5 days then $I_t = \frac{10}{2^{5/5}}$

$I_t = 5$

Example 2.

Half life = 5 days

Time elapsed = 8 days

I_o = 10 millicuries

$I_t = \frac{10 \text{ M.C.}}{2^{8/5}}$

$I_t = \frac{10}{2^{1.6}}$

$I_t = \frac{10}{\text{log of } 2^{.3 \times 1.6}}$ (log of 2 being obtained from slide rule)

$I_t = \frac{10}{\text{anti log } .48}$ or $\frac{10}{3.02}$ (anti log being obtained from slide rule)

I_t = 3.33 millicuries

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PART I

Charge electroscope to about 4.7 and start a background count when the cross bar has reached 5. Compute the time elapsed for the cross bar to travel between 5 and 6 and express results in divisions per second. This value might normally be anywhere from .001 to .005 divisions per second depending upon the sensitivity of the electroscope and the background from cosmic rays and the amount of radioactivity about the instrument.

PART II

Obtain a sample of P^{32} of known activity and a sample of I^{131} and determine rate of travel on electroscope. After the background is subtracted, this information can be used in the following manner:

a. The data is used on first point on a decay curve of P^{32} and I^{131}. Plot data on 3 cycle semi-log paper with days on linear scale, activity on log scale. Use this data to verify decay curve:

$$I_t = \frac{I_o}{2}^{\frac{\text{time elapsed}}{\text{half life}}}$$

b. Since standards furnished are calibrated in microcuries at the time first measurement was taken, it is possible to calibrate the particular electroscope used so that 1 microcurie is equal to x divisions per second, providing the geometry of sample is duplicated and both sources are of nearly the same energy.

PART III

Cross Calibration Counter and Electroscope

Obtain sample of P^{32} 1/100 times as active as that used with the electroscope. Run on Geiger counter and express results in counts per second. After background is subtracted, calculate value for 1 microcurie in counts per second. It can be seen that this value will be fairly constant for beta emittors providing the geometry is duplicated. Check results with a sample of radio-iodine of suitable activity for use in counter.

PART IV

Calculate how many counts per second on counter will equal one division per second on electroscope for phosphorus and iodine. It can be seen that the two instruments which have greatly different sensitivities can be used together in a quantitative manner.

EXERCISE II

a. Mass Absorption Curve.

1. Take sample of radioactive phosphorus which was used in Exercise 1 for electroscope calibration and place in standard position on the electroscope.

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2. Obtain a series of aluminum filters which alone or in combination with each other will give the following filter thicknesses: 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 50, 70, 100, 150, 200 mils. Measure area of each filter, weigh and calculate mass in milligrams cm^{-2}.

3. The background of the electroscope is determined. The sample to be used is then measured for activity at zero mass and after adding the filter combinations mentioned above. It can be seen after determinations are made that the value of the background becomes important as filter mass is increased to a large value. For this reason, the background must be accurately known.

4. Repeat procedure in Part III with I^{131} which is a beta and gamma emittor and with C^{14}.

5. After background has been subtracted, plot data on 3 cycle semi-log paper placing activities on log side, and mass increments on linear side. Compare curves.

b. Self Absorption.

Although mass absorption curves are useful for physical identification of various radio-elements, corrections for mass absorption in this manner in actual practice are rarely possible in the biological sciences. The mass of the system to be studied is usually intimately mixed with the radio-element used. It is therefore necessary to be able to correct for mass encountered during such studies. Because of the ease in obtaining thin films, either water is used or dilute acid to determine the change of measured activity as a function of mass of the sample.

1. Place a small amount of aerosol or liquid soap on a porcelain dish rubbing it over the surface of the dish carefully.

2. Obtain a solution of radio-phosphorus with sufficient activity so that 1/10 of one cc will be equivalent to approximately 80 counts per second on the counter.

3. Place 1/10 of one cc of the active solution into the dish making an effort, with the aid of the aerosol, to completely cover thedish with the film of the active solution.

4. Determine the total number of counts per second the sample gives.

5. Remove dish and add dilute acid so that before each measurement there will have been 0.2, 0.3, 0.5, 0.7, 1.0, 2.0, 3.0, 4.0, 5.0 grams of water in the dish. Mix the solution carefully by rotating the dish prior to each measurement. It will be seen that the apparent activity arising from the original sample is reduced each time additional mass is added. This situation being similar to that encountered when radioactive materials are incorporated in mass of biological origin. After the dish has been measured at a level which contain 5 grams, the entire material is evaporated to dryness on a hot plate below boiling and measured again for zero mass, care being taken to prevent boiling or spattering. Providing no material has been lost, this should be the same or slightly more active than

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the original 1/10 of a cc added. The data obtained is plotted on ordinary graph paper having the percent of the activity remaining on one side, the increments of mass on the other. This curve can be used to obtain the appropriate mass absorption corrections for any mass falling in the limits of the curve. A mass absorption curve must be obtained for each radio-element studied since the shape of the curve depends upon the energy of the radiations.

EXERCISE III.

1. Obtain a rat which has been given radio-phosphorus. Sacrifice the animal and determine its total weight. Dissect out heart, lungs, liver, spleen, kidneys, intestinal tract, sample of bone from femur of the right leg, dissect out the left leg entirely since this was the injection site, skin, muscle, and brain. Determine wet weight of these tissues, place them in suitable pyrex beakers, including the carcass of the animal and mark with ink which is designed to stand high temperatures. In order to reduce mass, the tissues are dried at 100 degrees Centigrade and the temperature is raised to about 200 degrees Centigrade for the following day. The tissues are then placed in an electric muffle and ashed at a temperature of 550 degrees for 24 hours, or until the residue remaining is nearly white ash. After the ashing is completed, the material is transferred to small metal dishes and spread evenly over the surface of the dishes so that adequate self-absorption corrections may be made.* The weight of ash is of course determined by weighing the dish empty and then after the ash has been added. At the same time the animals are injected, an accurately measured portion of the radioactive material administered to the rats is placed in a volumetric flask. Any known portion of this would represent an aliquot of the dose. The actual dose given can be determined each time tissues are assayed, the counts being expressed in counts per second or divisions per second, or both. Even if the tissues are run over a long period of time, any variations of the sensitivity of the measuring instrument or changes in geometry due to the replacement of a Geiger tube will be cancelled out if the aliquot of the dose is run at the same time as the tissues are run. Radioactive decay will also cancel itself out since it will be the same in the tissues and the portion of the dose. It is necessary, of course, to duplicate the geometry exactly when the samples are run and when the aliquot of the dose is run. If this is done then, and for example, say 1/100 of the dose has been found to be 30 counts per second after the background is subtracted from the determination, it follows then that the dose given the animal at this time and at this geometry would be 3000 counts per second. If the whole liver of the rat contained 100 counts per second after self-absorption corrections, it can be seen that the activity in the liver or other organs can be expressed in the total number of dose given, which in this case would be 3.33%. It is possible then to combine the activities determined on all of the samples so ashed, including the carcass and excrete, and determine the quantitative accuracy of the experiment.

* When the amount of ash is small, a drop of water will spread the ash out evenly on the dish. The water can be removed by drying on the hot plate at less than 100° C.

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2. Determine activity of dose. Assay tissues.

3. Prepare self absorption curve so that adequate mass absorption corrections can be made.

4. Compile data on table similar to that below.

Date	Background			C/S			% of Dose	
	Tissue	Wet Wt	Ash Wt	Counts Second	Mass Ab.	Corrected Counts	Gram	Organ
Heart								
Lungs								
Spleen								
Blood								
Liver								
Kidney								
Adrenals								
Thyroid								
Lymph gland								
Pancreas								
Brain								
Fat								
Teeth								
Stomach								
Small intestine								
Large intestine								
Bone								
Muscle								
Skeleton								
Balance								
Left leg								
Skin								
				Total % in Animal				
Urine								
Feces								
				Total % Recovered				
Aliquot of dose								

EXERCISE IV

The following experiment has been designed to deomonstrate the use of radioactive materials in one of the organ systems of the body involved in the physiological processes of secretion.

Obtain a female mouse which has recently had a litter and which was given radio-phosphorus (P^{32}) one day after the litter was born. In addition to this, obtain an aliquot of the dose given. The self absorption curve prepared in the previous exercise can be used for self absorption correction.

1. Each day after the start of the experiment, the young mice are

weighed. The area in square centimeters, which would be projected to the measuring instrument, is estimated and the appropriate mass absorption correction applied. Several of the mice are placed in suitable dishes and the activity determined in terms of the percent of the dose given the mother. This is done daily.

2. In addition to Part 1, at the end of the first, third, and fifth days of the experiment, the amount of activity is determined in one mouse in the litter, the mouse subsequently being sacrificed and ashed and the activity is measured so that a suitable check may be made on the self absorption correction which is made on the live animals. In addition to the above, each day an attempt should be made to obtain 1/10 of a cc of mouse milk using the technique which will be demonstrated. The activity of phosphorus per cc of milk being calculated from the results obtained from this sample. It is possible then to plot the data as follows:

a. Accumulation of radio-phosphorus per mouse and per litter during the course of the experiment as percent of the total dose given the mother.

b Retention of P^{32} per gram of baby mouse plotted against the time during which the experiment was continued.

c. Calculate the total number of cc's of milk porduced by the mouse as indicated by the phosphorus retnetion of the litter.

EXERCISE V
Tracer Studies Involving Iodine

1. Obtain a rat which has been injected with radio-iodine and an aliquot of the dose Sacrifice the animal and obtain the wet weight or a sample of the following organs: liver, 10 grams of muscle, femurs from both hind legs, kidneys, spleen and thyroid. After it is believed that the thyroid is completely removed, it is wise to check the larynx of the rat on the Geiger Muller counter in order to make certain that a portion of the thyroid tissue is not remaining there. Using an appropriate filter, count the gamma fraction of the radio-iodine of these samples only, the actual filter techniques having been determined by the mass absorption curve which was done on iodine earlier in the course, the aim being to absorb all beta radiation. Because of the activity involved, it is necessary to do this on the Geiger Muller counter. Calculate the results as a percent of dose in the organs mentioned above, per organ and per gram.

2. In order to separate the thyroxin, diiodo tyrosine, and iodide fractions of the thyroid, the following procedure is to be employed. Dissolve the entire thyroid in 20 cc's of two normal sodium hydroxide. Add approximately 10 milligrams of thyroid carrier as dessicated thyroid substance. This is then placed on the steam bath for 24 hours. Make volume of sodium hydroxide to 20 cc after steam bath treatment. Separate thyroid solution into 4 and 5 cc portions (one for each laboratory group member.) Of each 5 cc portion, take .5 cc's and place in a metal dish. This is evaporated on a hot plate at low and represents each group member's aliquot of the total thyroid sample.

3. Thyroxin fraction separation.

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Extract 4.5 cc fraction of Part 2 with two separate 10 cc portions of Butyl alcohol. Evaporate 1 cc of Butyl alcohol fraction on a metal dish, this representing 1/10 of the thyroxin present in total butyl alcohol fraction. Two extractions are necessary in order that the student may have some idea of the efficiency of the separation. Report efficiency of extraction.

4. Iodide fraction.:

Oxidize iodide ion to iodine by adding to a water phase remaining from the previous extraction, the following materials:

5 cc's of three normal hydrochloric acid.
1 cc and 20 milligrams of sodium iodide solution.
1 cc and 10 milligrams of sodium iodate solution.

Remove the oxidized ion (I_2) by extracting twice with 10 cc's of carbon tetrachloride. After the carbon tetrachloride has been separated from the aqueous phase, the carbon tetrachloride is shaken up with 10 cc's of 1/10 mole sodium thiosulphate containing .1 M NaOH. This reduces the iodine to the iodide state. Evaporate one cc of this solution upon a metal dish and determine the iodide fraction of the thyroid. Determine the activity remaining in 1 cc of the water phase resulting from the carbon tetrachloride extraction. This solution contains the diiodo tyrosine. With the data in hand, calculate the following:

a. Recovery of the total amount of iodine present in the thyroid in the three fractions discussed above. With good technique, this should be better than 90%.

b. In addition, calculate the percent of iodine in the three fractions discussed above.

EXERCISE VI
Radioautographs

Although normally radioautographs would be initiated by actually sectioning the tissues involved, the lack of facilities makes it necessary to use rather crude methods in preparation of the samples which are to be autographed.

1. Obtain a rat which has been given 1/4 millicurie of radio-phosphorus. Dissect out both femurs and remove mandible and split into two sections so that an incisor is in either half. Both the femur and the tooth preparation is to be prepared by sanding both sides down to a flat even plane by use of the emery paper which is supplied. This should be done on paper so that the rather radioactive dust resulting from the sanding can be disposed of without contaminating the laboratory. The bone should be ground so that the trabecular bone and the area around the epiphyseal can be clearly seen. With respect to the tooth preparation, they should be sanded down until the pulp cavity of the tooth is visible. It is worthwhile to leave the tooth in the surrounding bone so that a picture of the comparative deposition of radio-phosphorus can be seen between the bone and tooth structure. After the materials

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are sufficiently sanded and flat, the water is removed from the sections by emersing in alcohol ether mixture for 12 hours and allowing it to dry at room temperature for 4 to 5 hours. The sections are then placed over an electroscope and their activity measure, this being converted to total number of disintegrations per second per square centimeter arising from one plane of the section. Exposure time for the radio-autograph can be calculated by determining the time necessary to accumulate 5×10^6 disintegrations on the film per square centimeter. This can be calculated as follows:

$$\text{Exposure time in seconds } \frac{1 \times 10^7}{\text{microcuries CM}^{-2}} \times 3.7 \times 10^4$$

2. Radioautographs with plant material.

A plant will be furnished which has been grown in soil with a low phosphorus content. A known amount of radio-phosphorus is given to the plant along with 50 cc of water. Samples should be taken from the plant at about 14, 24, 48 and 72 hours after P^{32} administration to the soil. Samples should consist of new growth, old growth, stem, as well as seeds and flowers, if any.

The above materials are prepared for radio-autographs by placing a flat layer of scotch tape on both sides of the objects selected. The resulting preparation should be flat. Determine the disintegrations per second arising from the sections per square centimeter of area in order to calculate the exposure time. In addition to Part 1, take the strongest sample which has an exposure time of hours or minutes and vary the exposure time so that 1×10^6 disintegrations per second hit the film, as well as 5×10^6 and 1×10^7, in order to determine the effect of varying exposure time upon the clearness and quality of the radio-autograph.

3. Assay a similar portion of the tomato plant which was taken in Partl and express the results as a relative deposition in:

a. Growing tissues.

b. Conducting tissues such as the stem.

c. Old tissue such as the older leaves at the base of the plant.

d. A sample of the roots of the plant which have been well washed.

EXERCISE VII
Selective Accumulation if I^{131}
in the Thyroid
In Vivo Experiment

1. Obtain 20 microcuries of I^{131} and drink.

2. Immediately after administration of iodine, seat subject on a stool so that Geiger Muller counter measurements can be made in a comfortable manner on both the region around the larynx at the front of

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neck, and a similar region at back of neck at the same level. The window of the counter should be placed about 1 cm away from the subject.

3. Count front and back of neck at five minute intervals (see note).

4. Obtain an aliquot of the dose in a bottle which reproduces geometry of iodine deposition in the thyroid with respect to mass absorption. Calculate dose in counts per second in this manner.

5. Collect 24 hour urine and assay gamma activity to determine percent of dose excreted. Repeat for 3 days.

6. Plot half time of I in body not absorbed by thyroid.

7. Report percent of I uptake by thyroid.

Note:

During early measurements right after the administration of iodine, the activity emanating from the region of the thyroid can concist of (a) Activity in the circulating blood of the body, unabsorbed I^{131} in the stomach, liver, muscle, etc., and (b) activity deposited in the thyroid. It can be seen that measuring the activity at the front of the neck and comparing it to that at the back of the neck would introduce a variable in geometry of the activity coming from the thyroid. This would not be true as far as Part (a) is concerned, however, since the distance of most of the body from the counter is approximately the same at the fron and back, hence the geometry is the same. Iodine rapidly leaves the body and it can be seen then that after 24 hours, the activity emanating from the front or the back of the neck is due primarily to the location of iodine in the thyroid.

This latter measurement may be made and, for example, it is found that 15% of the activity observed in the frontis measured from the back of the neck. During the early measurement then, any dissimilarity between the front and back measurements will be contributed by the thyroid. For example, if the front of the neck give 100 c/s and the back 50 c/s, the difference of 50 counts per second is equal to 15% of the activity of the thyroid plus the background from the rest of the body. (See J G Hamilton and Mayo H. Soley studies on iodine metabolism of the thyroid gland in situ by the use of radio-iodine in normal subject and in patients with various types of goiter. The Amer. Jour. of Physiology, Vol. 131, No. 1, Nov. 1940.)