November 1, 1950

MEMORANDUM

TO: Dr. D. W. Colvard, Head
Department of Animal Industry

FROM: George H. Wise

SUBJECT: Proposed project on metabolism of lipids.

Attached hereto is a proposed project which we shall appreciate your transmitting to Doctor Cummings if the outline meets with your approval.

Some time ago when Doctors Tove and Matrone met with Doctors Anderson and Cummings to discuss the current project in the use of radioactive copper, the possibilities of getting support for the type of work outlined in the attached project was considered. Doctor Cummings suggested that a tentative outline be submitted to his office for review by an Experiment Station committee to ascertain whether or not the type of investigation proposed would be suitable to submit to the Atomic Energy Commission for consideration. The hope is that the plans merit attention by the Atomic Energy Commission and that support through this agency may be obtained. The proposed budget is somewhat small, but at this stage we are at a loss to know what to recommend specifically.

If you have any suggestions for amending the outline before transmission to Doctor Cummings, please let us know.

GHW/di

Enclosures/4
cc: Dr. Tove

November 1, 1950

MEMORANDUM:

TO: Dr. D. W. Colvard, Head
Department of Animal Industry

FROM: George H. Wise

SUBJECT: Proposed project on metabolism of lipids.

Attached hereto is a proposed project which we shall appreciate your transmitting to Dr. Cummings if the outline meets with your approval.

Some time ago when Doctors Tove and Matrone met with Doctors Anderson and Cummings to discuss the current project in the use of radioactive copper, the possibilities of getting support for the type of work outlined in the attached project was considered. Dr. Cummings suggested that a tentative outline be submitted to his office for review by an Experiment Station committee to ascertain whether or not the type of investigation proposed would be suitable to submit to the Atomic Energy Commission for consideration. The hope is that the plans merit attention by the Atomic Energy Commission and that support through this agency may be obtained. The proposed perhaps is somewhat bold, but at this stage we are at a loss to know what to recommend specifically.

If you have any suggestions for amending the outline before transmission to Dr. Cummings, please let us know.

GHW/di
Enclosure/4
cc: Dr. Tove

Dr. Wise.

1st [revised 12-28-50]

PROPOSED RESEARCH PROJECT OUTLINE

1. Title: Requirement and metabolism of lipids as influenced by the composition of the diet.

2. Objectives: To determine how the metabolism of lipids is affected by the level of fat and vitamins in the diet of the various laboratory animals and classes of livestock.

3. Reasons for undertaking investigations:

Since fat formation is one of the principal factors involved in the production of marketable livestock, and one of the prime considerations in milk production, it is important to know how diet composition can affect this process. A better understanding of fat metabolism in animals should effect more efficient nutrition of livestock.

4. Previous work and present status:

Although it generally is recognized that the composition of dietary fat and levels of other nutrients will affect the nature and amount of fat produced by an animal, there are large gaps in the knowledge of the mechanisms involved. The metabolism of fat is a complicated process which undoubtedly depends on many metabolic pathways in the animal body. There is a need for more specific information on the factors which affect fat metabolism and their mechanism of action.

5. Outline of procedure:

The general procedure to be followed is one of using the laboratory animal as a guide, and then study the more promising leads in livestock. In order to control the diets of the experimental animals as closely as possible, purified diets will be used wherever feasible.

Subproject A

1. Subtitle: Lipid metabolism in the rat as affected by composition of the diet.

2. Objectives: To determine if a deficiency of one or more of the following factors affects the rate of either synthesis or catabolism of fatty acids, cholesterol and phospholipids: linoleic acid, biotin, pyridoxine, pantothenic acid and choline.

3. Reasons for undertaking investigations:

Although all of the aforementioned dietary factors have been implicated in lipid metabolism, their exact role (if there be one) is

still undetermined. The rat has been chosen as the test animal in the interest of experimental efficiency and because this animal has been used to a large extent in previous studies on fat metabolism.

4. Previous work and present status:

Although twenty years have passed since Burr and Burr (J. Biol. Chem. 86:618, 1930) observed that a dermititis that rats develop on a fat-free diet can be cured by small amounts of linoleic or arachidonic acids, the specific function of these compounds is unknown. It has been assumed, generally, that linoleic acid and arachidonic acid were concerned with the formation of phospholipids and that the latter compounds were important in the metabolism of fat. On the other hand, Smedley-Maclean and Nunn (Biochem. J. 34: 884, 1940) have shown that in an essential fatty acid deficiency there is no decrease in liver phospholipids and that muscle phospholipids increased when linoleic acid was decreased from one drop to one-half or one-fourth drop per rat per day.

It has been established that biotin can replace oleic acid for the growth of lactic acid bacteria (J. Biol. Chem. 169:761, 1947; 170: 619, 1947; 172:531, 1948). Gavin and McHenry (J. Biol. Chem. 141: 619, 1941) have shown that under certain conditions biotin can cause fatty livers and an increase in the amount of C_{18} fatty acids as well as unsaturated fatty acids. It thus would appear likely that biotin functions in the synthesis of fatty acids in animals as well as in bacteria. This hypothesis is to be tested.

Several workers have shown that pyridoxine is required for the complete cure of the syndrome described by Burr and Burr (J. Biol. Chem. 124:775, 1938; 132:41, 1940; 140:CIX, 1941). Although this vitamin is known to be involved in amino acid metabolism, apparently it has not been studied as functioning in fat metabolism.

Pantothenic acid has been shown to be part of coenzyme A, which is associated with the metabolism of acetate (J. Biol. Chem. 160:173, 1945; 167:869, 1947). Since it is known that fatty acids are synthesized from acetate and broken down via acetate, one would expect a pantothenic acid deficiency to have a marked effect on fat metabolism.

Since choline is part of the molecule of phospholipids and these latter compounds are concerned with fat metabolism, this vitamin should have an effect on the rate of formation and/or breakdown of fat. A choline deficiency would be useful in ascertaining whether any observed changes in fat metabolism are due to lack of synthesis of fatty acids or lack of phospholipid formation and reduced mobility of fat from one part of the body to another.

5. Outline of procedure:

The general procedure involves feeding to rats a purified diet

deficient in one or more of the factors to be studied. When characteristic deficiency symptoms appear, the animals will be injected with C^{14} labeled acetate (carboxyl labeled). This compound will be prepared from C^{14}-$BaCO_3$ by carbonation of CH_3MgI. After a suitable period, to be determined by pilot investigations, the rats will be sacrificed and the liver and carcass fat removed by solvent extraction. The phospholipids will be separated by acetone precipitation and the remaining fat saponified. Cholesterol will be separated by digitonin precipitation, and the unsaturated fatty acids removed by bromination. The saturated fatty acids will be separated by chromatography following the procedure of Howard and Martin (Biochem. J. 46: 532, 1950). The amount of C^{14} in each of the isolated compounds will be counted. This will give a measure of either the rate of synthesis or rate of catabolism depending on the time elapsing between the injection of radioacetate and death.

The basal diet will consist of:

Sucrose - - - - - - -	76
Vitamin-free casein - -	20
Salts - - - - - - - -	4
Cystine - - - - - - -	0.2
Methyl Linoleate - - -	0.1 gm/day given by dropper.

The following vitamins will be added in mg/100 gm ration:

Thiamin	0.2	Biotin	0.01
Riboflavin	0.3	Folic acid	0.2
Pyridoxine	0.25	Menadione	0.5
Ca pantothenate	2.0	Inositol	10.0
Niacin	1.0	Vitamin B_{12}	0.005
Choline	100.0		

Vitamins A, D and E to be given twice weekly in methyl laurate.

The following diets will be tested:

1. Basal
2. Basal less methyl linoleate
3. Basal less choline
4. Basal less pantothenic acid
5. Basal less pyridoxine
6. Basal less methyl linoleate and choline
7. Basal less methyl linoleate and pyridoxine
8. Egg white basal (egg white replacing casein)
9. Egg white basal less biotin
10. Egg white basal less biotin and methyl linoleate
11. Egg white basal less methyl linoleate

During the period after injection of the radioacetate and death, the rats will be placed in metabolism cages in a hood so that expired radiocarbon will be carried away by ventilation. Disposal of carcasses containing radiocarbon will be carried out according to the recommendations of the isotope committee of North Carolina State College.

A pilot experiment will be conducted on thirty rats using diets 1, 4 and 8. The purpose of this pilot experiment will be to determine the turnover rate of fatty acids, cholesterol and phospholipids under the proposed experimental conditions and to serve as a basis for the efficient planning of future experiments.

In addition, if time and preliminary experiments indicate it to be of value, the rate of oxidation of fat by isolated liver enzyme systems from rats on the various deficiencies will be determined.

6. Probable Duration: Three years

7. Date of Initiation: January 1, 1951

8. Personnel:

Name	Department	Relation to Project
S. B. Tove	Animal Industry	Leader
F. W. Sherwood	Animal Industry	Co-leader
F. H. Smith	Animal Industry	Co-leader
G. H. Wise	Animal Industry	Adviser
H. L. Lucas	Statistics	Adviser

9. Cooperation:

Interdepartmental; Institute of Statistics

10. Proposed Budget:

1951.....	$2000
1952.....	1500
1953.....	1500
	$5000

Scope and Purpose

There is little direct evidence concerning the exact metabolic pathways by which lipides are synthesized and degraded in animal tissues. This is largely because these processes are intimately associated with a series of complex oxidative and phosphorylative systems in which the individual metabolic reactions are difficult to separate and study. It is well established that a vitamin deficiency results in a block of the metabolic pathway in which this nutrient is involved, although these blocks are often difficult to detect. It is the purpose of this project to determine if the rate of synthesis or catabolism of fatty acids, phospholipids and cholesterol is altered by a deficiency of one or more of the following dietary essentials (all of which have been implicated in lipide metabolism): pantothenic acid, biotin, pyridoxine, choline and linoleic acid. The sensitive isotopic tracer technique will be used to detect any metabolic changes which occur. Should such changes become apparent, an attempt will be made to determine the mechanism by which the factor affects lipide metabolism. Furthermore, interrelationships between those dietary factors which appear to be involved in lipide metabolism will be studied.

North Carolina State College of Agriculture and Engineering
of the
University of North Carolina
Raleigh
STATE COLLEGE STATION

SCHOOL OF AGRICULTURE AND FORESTRY
AGRICULTURAL EXPERIMENT STATION
AGRICULTURAL EXTENSION SERVICE
RESIDENT TEACHING

DEPARTMENT OF ANIMAL INDUSTRY

6 December 1950

MEMORANDUM TO: Dr. G. H. Wise

I am returning herewith the proposed research project outline dealing with the metabolism of lipids. Dr. Cummings has proposed that this be written as one project without the subproject heading. Inasmuch as support may be sought on a contract basis it was his belief that it would be better to submit the project as a complete proposal to which funds could be allocated and the work completed rather than to suggest that other phases would be developed later.

I do not believe this will be difficult to do. You may want to combine your objectives and reasons for undertaking investigations, using your general objectives and following with either specific objectives or simply adding to the broad statements made on the first page.

I would like to suggest that you recalculate the proposed budgets, giving some indication of the purpose for which funds would be used. I think it would be well in this connection to include at least a part of the salary of one or more persons and also to be sure that the budget requested is adequate to cover the work you have in mind.

When this project has been developed along these lines you can re-submit it for review.

D. W. Colvard, Head
Department of Animal Industry

DWC:ho

December 30, 1950

MEMORANDUM

TO: Dr. D. W. Colvard, Head
Department of Animal Industry

FROM: George H. Wise

SUBJECT: Outline of proposed research project pertaining to the effect of composition of the diet on lipide metabolism

I am returning herewith a revised outline for the project that we would like to have considered with the view of submission to the Atomic Energy Commission. I believe that the delineation of the work is indicated sufficiently well to warrant consideration by A.E.C.

Inasmuch as Dr. Tove will be leaving for Oak Ridge within about a week, I shall appreciate your submitting this to Dr. Cummings as soon as possible.

GHW/di
Enclosure

2 nd
Revised 12/28/60

PROPOSED RESEARCH PROJECT OUTLINE

1. Title: Effect of composition of the diet on lipide metabolism

2. Objectives: To determine whether or not the rate of either synthesis or catabolism of fatty acids, cholesterol and phospholipides is affected by a deficiency of any of the following factors: linoleic acid, biotin, pyridoxine, pantothenic acid and choline

3. Reasons for undertaking investigations:

Although all of these dietary factors have been implicated in lipide metabolism their exact role (if there be one) is still undetermined.

4. Previous work and present status:

Although twenty years have passed since Burr and Burr (J. Biol. Chem. 86:618, 1930) observed that a dermititis that rats develop on a fat-free diet can be cured by small amounts of linoleic or arachidonic acids, the specific function of these compounds is unknown. It has been assumed, generally, that linoleic acid and arachidonic acid were concerned with the formation of phospholipids and that the latter compounds were important in the metabolism of fat. On the other hand, Smedley-Maclean and Nunn (Biochem. J. 34:884, 1940) have shown that in an essential fatty acid deficiency there is no decrease in liver phospholipids and that muscle phospholipids increased when linoleic acid was decreased from one drop to one-half or one-fourth drop per rat per day.

It has been established that biotin can replace oleic acid for the growth of lactic acid bacteria (J. Biol. Chem. 169:761, 1947; 170: 619, 1947; 172:531, 1948). Gavin and McHenry (J. Biol. Chem. 141: 619, 1941) have shown that under certain conditions biotin can cause fatty livers and an increase in the amount of C_{18} fatty acids as well as unsaturated fatty acids. It thus would appear likely that biotin functions in the synthesis of fatty acids in animals as well as in bacteria. This hypothesis is to be tested.

Several workers have shown that pyridoxine is required for the complete cure of the syndrome described by Burr and Burr (J. Biol. Chem. 124:775, 1938; 132:41, 1940; 140:CIX, 1941). Although this vitamin is known to be involved in amino acid metabolism, apparently it has not been studied as functioning in fat metabolism.

Pantothenic acid has been shown to be part of coenzyme A, which is associated with the metabolism of acetate (J. Biol. Chem. 160:173, 1945; 167:869, 1947). Since it is known that fatty acids are synthesized from acetate and broken down via acetate, one would expect a pantothenic acid deficiency to have a marked effect on fat metabolism.

Since choline is part of the molecule of phospholipids and these latter compounds are concerned with fat metabolism, this vitamin should have an effect on the rate of formation and/or breakdown of fat. A choline deficiency would be useful in ascertaining whether any observed changes in fat metabolism are due to lack of synthesis of fatty acids or lack of phospholipid formation and reduced mobility of fat from one part of the body to another.

5. Outline of procedure:

The general procedure involves feeding to rats a purified diet deficient in one or more of the factors to be studied. When characteristic deficiency symptoms appear, the animals will be injected with C^{14} labeled acetate (carboxyl labeled). This compound will be prepared from $C^{14}BaCO_3$ by carbonation of CH_3MgI. After a suitable period, to be determined by pilot investigations, the rats will be sacrificed and the liver and carcass fat removed by solvent extraction. The phospholipids will be separated by acetone precipitation and the remaining fat saponified. Cholesterol will be separated by digitonin precipitation, and the unsaturated fatty acids removed by bromination. The saturated fatty acids will be separated by chromatography following the procedure of Howard and Martin (Biochem. J. 46: 532, 1950). The amount of C^{14} in each of the isolated compounds will be counted. This will give a measure of the rate of synthesis of these metabolites. The rate of catabolism of the foregoing lipides will be determined by the use of enzyme preparations of surviving rat liver.

The basal diet will consist of:

Sucrose	76
Vitamin-free casein	20
Salts	4
Cystine	0.2
Methyl Linoleate	0.1 gm/day given by dropper

The following vitamins will be added in mg/100 gm ration:

Thiamin	0.2	Biotin	0.01
Riboflavin	0.3	Folic acid	0.2
Pyridoxine	0.25	Menadione	0.5
Ca pantothenate	2.0	Inositol	10.0
Niacin	1.0	Vitamin B_{12}	0.005
Choline	100.0		

Vitamins A, D and E to be given twice weekly in methyl laurate.

The following diets will be tested:

1. Basal
2. Basal less methyl linoleate
3. Basal less choline
4. Basal less pantothenic acid

5. Basal less pyridoxine
6. Basal less methyl linoleate and choline
7. Basal less methyl linoleate and pyridoxine
8. Egg white basal (egg white replacing casein)
9. Egg white basal less biotin
10. Egg white basal less biotin and methyl linoleate
11. Egg white basal less methyl linoleate

During the period between injection of the radioacetate and death, the rats will be placed in metabolism cages in a hood so that expired radiocarbon will be carried away by ventilation. Disposal of carcasses containing radiocarbon will be carried out according to the recommendations of the isotope committee of North Carolina State College.

A pilot experiment will be conducted on thirty rats using diets 1, 4 and 8. The purpose of this pilot experiment will be to determine the turnover rate of fatty acids, cholesterol and phospholipids under the proposed experimental conditions and to serve as a basis for the efficient planning of future experiments.

6. Probable Duration: Three years

7. Date of Initiation: January 1, 1951

8. Personnel:

Name	Department	Relation to Project
S. B. Tove	Animal Industry	Leader
F. W. Sherwood	Animal Industry	Co-leader
F. H. Smith	Animal Industry	Co-leader
C. H. Wise	Animal Industry	Adviser
H. L. Lucas	Institute of Statistics	Adviser

9. Cooperation:

Interdepartmental; Institute of Statistics

10. Budget Breakdown:

1951: -- Personnel - Graduate Assistant ---	$ 1500	
Animals ----------	200	
Rations ----------	450	
Isotopes ----------	350	
Chemicals and glassware, misc. equipment	500	
		$ 3000
1952: -- Personnel - Graduate Assistant --	1500	
Animals ----------	200	
Rations ----------	450	
Chemicals, glassware, misc. equip.	350	
		2500
1953: Same as 1952 ----------		2500
Total:		$ 8000

April 28, 1951

U. S. Atomic Energy Commission
Washington 25, D. C.

Dear Sirs: Attention: Dr. Paul B. Pearson, Chief, Biological Branch, Division of Biology and Medicine

Five copies of a proposed project entitled, "Effect of the Composition of the Diet on Lipide Metabolism" are submitted herewith for your consideration. This proposal, as indicated by the accompanying letter from Dr. R. W. Cummings, Director of Research, is in accord with the program of the North Carolina Agricultural Experiment Station.

The procedures have been reviewed and approved by Dr. Newton Underwood, Radiological Safety Officer of the Radioisotope Committee of North Carolina State College.

I trust that the proposed project may merit your favorable consideration.

Very truly yours,

George H. Wise, Head
Animal Nutrition Section

GHW/di

North Carolina State College of Agriculture and Engineering
of the
University of North Carolina
Raleigh

SCHOOL OF AGRICULTURE
RESEARCH EXTENSION
RESIDENT TEACHING

AGRICULTURAL EXPERIMENT STATION

OFFICE OF DEAN AND DIRECTOR

April 28, 1951

U. S. Atomic Energy Commission
Washington 25, D. C.

Attention: Dr. Paul B. Pearson, Chief, Biology Branch
Division of Biology and Medicine

Dear Sir:

Our Department of Animal Industry has prepared a project proposal on "Effect of the Composition of the Diet on Lipide Metabolism" which they are submitting to you with a request for financial support. We would be very much interested in following through on such a study and sincerely hope that the proposal will meet with your favorable consideration.

Very truly yours,

R. W. Cummings
Director of Research.

File: Animal Nutrition
3rd + Final revision
4/28/51

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
DEPARTMENT OF ANIMAL INDUSTRY
N. C. State College
Raleigh

PROPOSED RESEARCH PROJECT OUTLINE

(1) TITLE: Effect of the composition of the diet on lipide metabolism.

(2) OBJECTIVES: 1. - To determine whether the rate of either synthesis or catabolism of fatty acids, cholesterol and phospholipides is affected by a deficiency of one or more of the following dietary essentials: linoleic acid, biotin, pantothenic acid, pyridoxine and choline.

2. - To determine the mechanism by which lipide metabolism is altered by a deficiency of the aforementioned factors, should any metabolic changes become apparent.

3. - To explore interrelationships between those dietary essentials which appear to be involved in lipide metabolism.

(3) REASONS FOR UNDERTAKING INVESTIGATIONS:

The basic program of the Animal Nutrition Section of the Department of Animal Industry is to investigate fundamental problems relating to the nutrition and metabolism of animals with the ultimate aim of applying any information gained to livestock production. The proposed project is in keeping with this policy.

There is little direct evidence concerning the exact metabolic pathways by which lipides are synthesized and degraded in animal tissues. This is largely due to the fact that these processes are intimately associated with a series of complex oxidative and phosphorylative systems. Inasmuch as it has not been possible to separate and study the individual metabolic reactions of these systems, the use of experimental procedures that proved to be valuable in elucidating the pathways of carbohydrate metabolism have not been applicable. It is well established that a vitamin deficiency results in a block of the metabolic pathway in which this nutrient is involved; although these blocks are often difficult to detect by the usual analytical techniques of the biochemist. The great selectivity and sensitivity inherent in the tracer technique overcomes many of these difficulties and, thus, the use of isotopic tracers becomes a virtual necessity in the study of lipide metabolism. Since all of the aforementioned vitamins have been implicated in lipide metabolism (see below), it is presumed that a deficiency of one or more of these factors would produce a metabolic block detectable by the sensitive tracer technique. If the foregoing hypothesis is valid, such an experimental approach should permit a more detailed study of lipide metabolism.

(4) PREVIOUS WORK AND PRESENT STATUS:

Although twenty years have passed since Burr and Burr (J. Biol. Chem. 86:618, 1930) observed that a dermititis which rats develop on a fat-free diet can be cured by small amounts of linoleic or arachidonic acids, the specific function of these compounds is unknown. It has been assumed, generally, that linoleic acid and arachidonic acid were concerned with the formation of phospholipides and that the latter compounds were important in the metabolism of fat. On the other hand, Smedley-Maclean and Nunn (Biochem. J. 34:884, 1940) have shown that in an essential fatty acid deficiency there is no decrease in liver phospholipides and that muscle phospholipides increased when linoleic acid was decreased from one drop to one-half or one-fourth drop per rat per day.

It has been established that biotin can replace oleic acid for the growth of lactic acid bacteria (J. Biol. Chem. 169:761, 1947; 170: 619, 1947; 172:531, 1948). Gavin and McHenry (J. Biol. Chem. 141: 619, 1941) have shown that under certain conditions biotin can cause fatty livers and an increase in the amount of C_{18} fatty acids as well as unsaturated fatty acids. Thus, it would appear likely that biotin functions in the synthesis of fatty acids in animals as well as in bacteria. This hypothesis is to be tested.

Several workers have shown that pyridoxine is required for the complete cure of the syndrome described by Burr and Burr (J. Biol. Chem. 124: 775, 1938; 132:41, 1940; 140:CIX, 1941). Although this vitamin is known to be involved in amino acid metabolism, apparently its role in fat metabolism has not been investigated.

Pantothenic acid has been shown to be part of coenzyme A, which is associated with the metabolism of acetate (J. Biol. Chem. 160:173, 1945; 167:869, 1947). Since it is probable that fatty acids are synthesized from acetate and broken down via acetate, one would expect a pantothenic acid deficiency to have a marked effect on fat metabolism. A recent paper (Fed. Proc. 10:226, 1951) suggests that pantothenic acid is required for the synthesis of cholesterol.

Since choline is part of the molecule of phospholipides and these latter compounds are concerned with fat metabolism, this vitamin should have an effect on the rate of formation and/or breakdown of fat. A choline deficiency would be useful in ascertaining whether any observed changes in fat metabolism are due to lack of synthesis of fatty acids or lack of phospholipide formation and reduced mobility of fat from one part of the body to another.

(5) OUTLINE OF PROCEDURE:

The general procedure involves feeding to rats a purified diet deficient in one or more of the factors to be studied. When characteristic deficiency symptoms appear, the animals will be injected with C^{14} labeled acetate (carboxyl labeled). After a suitable period, to be determined

by pilot investigations, the rats will be sacrificed and the liver and carcass fat removed by solvent extraction. The phospholipides will be separated by acetone precipitation and the remaining fat saponified. Cholesterol will be separated by digitonin precipitation, and the unsaturated fatty acids removed by bromination. The saturated fatty acids will be separated by chromatography following the procedure of Howard and Martin (Biochem. J. 46:532, 1950). The amount of C^{14} in each of the isolated compounds will be counted. This will give a measure of the rate of synthesis of these metabolites. In addition the rate of synthesis of fatty acids, phospholipides and cholesterol will be studied in vitro. Tissue slices and washed tissue homogenates will be used in these studies. Rate of catabolism will be studied by the addition of suitable radioactive metabolites to tissue preparations such as described.

The basal diet will consist of:

Sucrose	76
Vitamin-free casein	20
Salts	4
Cystine	0.2
Methyl linoleate	0.1 gm/day given by dropper.

The following vitamins will be added in mg./100 gm. ration:

Thiamin	0.2	Biotin	0.01
Riboflavin	0.3	Folic acid	0.2
Pyridoxine	0.25	Menadione	0.5
Ca pantothenate	2.0	Inositol	10.0
Niacin	1.0	Vitamin B_{12}	0.005
Choline	100.0		

Vitamins A, D and E are to be given twice weekly in methyl laurate.

The following diets will be tested:

1. Basal
2. Basal less methyl linoleate
3. Basal less choline
4. Basal less pantothenic acid
5. Basal less pyridoxine
6. Basal less methyl linoleate and choline
7. Basal less methyl linoleate and pyridoxine
8. Egg white basal (egg white replacing casein, biotin injected)
9. Egg white basal less biotin
10. Egg white basal less biotin and methyl linoleate
11. Egg white basal less methyl linoleate

During the period between the injection of the radioacetate and death, the rats will be placed in metabolism cages in a hood. There will be no more than four animals in the hood at any one time. The maximum dose any rat will receive will be 50 uc of C^{14}. The hood has a forced draft of 1300 cu. ft. of air per minute and is vented directly through the roof of the building. Thus, if 200 uc of radiocarbon were expired

by the rats within one minute, the concentration in the air leaving the hood would be 0.53×10^{-5} uc per cc. of air. This is approximately half the concentration of radiocarbon designated as safe for human respiration by the Atomic Energy Commission (AEC Isotopes Division Circular B-5). All carcasses, excreta and contaminated matter will be disposed of by incineration in the presence of an excess of carbonaceous matter. Separation of C^{14} containing compounds will be carried out in a closed system. All of the components to be separated are very slightly volatile.

A pilot experiment will be conducted on thirty rats using diets 1, 4 and 8. The purpose of this pilot experiment will be to determine the turnover rate of fatty acids, cholesterol and phospholipides under the proposed experimental conditions and to serve as a basis for the efficient planning of future experiments.

(6) PROBABLE DURATION: Three years

(7) DATE OF INITIATION: July 1, 1951

(8) PERSONNEL:

Name	Department	Relation to Project
S. B. Tove	Animal Industry	Leader
F. W. Sherwood	Animal Industry	Co-leader
F. H. Smith	Animal Industry	Co-leader
N. Underwood	Physics	Cooperator
G. H. Wise	Animal Industry	Adviser
H. L. Lucas	Institute of Statistics	Adviser
C. K. Beck	Physics	Adviser

PERSONAL RECORD:

Samuel B. Tove, Department of Animal Industry, Assistant Research Professor; received B. S. from Cornell University in 1943, M. S. From the University of Wisconsin in 1948 and Ph.D. from the University of Wisconsin in 1950. He was a member of the eighteenth class of the Oak Ridge Institute of Nuclear Studies Radioisotope Technique course. He has several publications dealing with unidentified factors required by the mink. His membership in professional and scientific societies include: Society of the Sigma Xi, Phi Lambda Upsilon, the American Chemical Society, the American Association for the Advancement of Science and the North Carolina Academy of Science.

Francis W. Sherwood, Department of Animal Industry, Professor; received his B.S. from N. C. State College in 1909, his M.S. from Cornell University in 1911 and his Ph.D. from Cornell University in 1921. He was Assistant in Soils, Cornell University 1912-1913; Assistant in Organic Chemistry, Cornell University, 1913-1916; Research Chemist, Federal Dyestuff and Chemical Corporation, 1916-1917; Research on poison gas, Chemical Warfare Service, U. S. Army, 1917-1918, Research Chemist, E. I. Dupont deNemours Company, 1919; Assistant Professor of Animal Nutrition, North Carolina State College Agricultural Experiment Station, 1919-1930, Associate Professor, Animal

Nutrition, 1930-1945, Professor, Animal Nutrition, 1945 to date. Dr. Sherwood has several publications relating to the use of animals for vitamin assay, and vitamin and mineral content of various feedstuffs. His membership in professional and scientific societies include: Society of the Sigma Xi, Alpha Chi Sigma, American Chemical Society, American Association for the Advancement of Science, North Carolina Academy of Science and Gamma Alpha.

Frank H. Smith, Department of Animal Industry, Associate Research Professor. Received B.S. from Davidson College in 1926, M.S. from North Carolina State College in 1931. He was Junior Chemist with the North Carolina State Department of Agriculture for the year 1927; Assistant Research Professor, Animal Nutrition, North Carolina Agricultural Experiment Station, 1928-1946; and Associate Research Professor in Animal Nutrition, 1947 to date. Mr. Smith has publications on the estimation of gossypol in cottonseed products, mineral content of forage crops, rancidity in bacon, curing of hams. He is a member of the Society of the Sigma Xi, American Oil Chemist Society, American Chemical Society and the North Carolina Academy of Science.

George H. Wise, Head of the Animal Nutrition Section of the Department of Animal Industry. He received his B.S. from Clemson Agricultural College in 1930, his M.S. from the University of Minnesota in 1932 and his Ph.D. from the University of Minnesota in 1936. He has held the following positions: Associate Dairy Husbandman, Clemson Agricultural College, 1937-1944; Associate Professor of Dairy Husbandry, Kansas State College, 1944-1947; Associate Professor of Dairy Husbandry, Iowa State College, 1947-1949; Professor of Animal Industry, North Carolina State College, 1949 to date. Dr. Wise has many publications covering nutrition and physiology of dairy cattle. His membership in scientific and professional societies include: Society of the Sigma Xi, American Dairy Science Association, the American Association for the Advancement of Science, American Society of Animal Production.

Henry L. Lucas, Institute of Statistics, Professor. Received his B.S. in Animal Husbandry, University of California, 1937; Ph.D. in Animal Nutrition, Cornell University, 1943. He has held the following positions: Supervisor Advanced Registry Cow Testing, California, 1938; Asst. in Animal Nutrition, University of California, 1938-39; Dairy Cattle Feed Investigator, Cornell, 1940-43; Research Associate in Animal Nutrition, Cornell, 1943; Instructor in Animal Physiology, Cornell, 1944; Research Associate in Poultry Nutrition, Cornell, 1944-45; Associate Professor of Statistics, N. C. State College, 1946-48; Professor of Statistics, N. C. State College, 1948 to date. His professional societies include A.A.A.S., American Society of Animal Production, American Dairy Science Association, American Statistical Association and Biometric Society; Honor societies include, Society of the Sigma Xi, Phi Kappa Phi, and Alpha Zeta. He has many publications in fields of Dairy Cattle and Poultry Nutrition, The Design of Animal Experiments and the Analysis of Data from Animal Experiments.

(9) COOPERATION: Interdepartmental - Department of Animal Industry, Institute of Statistics and Physics Department
Other agencies: United States Atomic Energy Commission

(10) BUDGET BREAKDOWN:
Financial Support

Funds requested:-

First year —	Personnel - Graduate Assistant - Salaries One	$ 1,500	
	Animals	200	
	Rations	550	
	Equipment (refrigerated centrifuge)	2,000	
	Isotopes	550	
	Supplies	200	
			$5,000
Second year -	Personnel - Graduate Assistant	1,500	
	Animals	200	
	Rations	550	
	Supplies	250	
			2,500
Third year —	Same as 1952 (or second year)		2,500
		Total:	$ 10,000

See attached sheet

Provided by North Carolina State College during the three-year period:

Personnel - Salaries		
S. B. Tove, leader, one-half time	$ 7,500	
Other listed project personnel	6,500	
Animal caretakers and misc. labor	1,500	
Equipment and Supplies		
Warburg Barcroft respiration apparatus and accessories	1,500	
Automatic fraction collector	500	
Animal cages	500	
Radioactivity assay equipment	1,000	
Special glassware	250	
Analytical instruments (Microbalance, photoelectric colorimeter, automatic titrator, pH meter)	2,500	
Miscellaneous glassware and reagents	500	
		6,750
Total:		$ 22,250

AVAILABLE FACILITIES:

The experimental animals will be housed in a large animal room situated on the third floor of the Animal Industry Building. Those animals receiving injections of radiocarbon will be placed in a hood designed according to AEC recommendations. The department has a Warburg Barcroft microrespiration apparatus for use in the in vitro studies. An automatic fraction collector is available for the chromatographic separation of the fatty acids. The institution has an air conditioned, constant temperature counting room which will be used. Such analytical and radioactive assay equipment as required for this project is available on the campus.

UNITED STATES
ATOMIC ENERGY COMMISSION

GC:JN

Oak Ridge, Tennessee
June 29, 1951

North Carolina State College
Department of Animal Industry
Raleigh, North Carolina

Attention: Dr. S. B. Tove

Subject: CONTRACT NO. AT-(40-1)-1324

Dear Dr. Tove:

Your research project which was submitted to the Commission's Division of Biology and Medicine, Washington, D. C., has been approved by that office in the amount of $5,000.00 and has been forwarded to this office for preparation of an appropriate contract covering the Commission's support of your project.

Enclosed in four copies, duly executed on behalf of the Commission, is a contract numbered as shown in the subject line above, which incorporates in Appendix "A" a description of your project and the budget for the first period which you are to follow as a general guide.

It is requested that you sign each copy of the contract in the space provided for the Project Leader on the signature page of the contract and have the contract signed by the proper official of the College, returning the original and one copy to this office. The two remaining copies are for your retention.

It will be noted that the contract provides for payment in Article III of a lump sum in consideration of your performance of the research activities described in Appendix "A". The first payment, representing one-half the amount of the agreed compensation, will be paid to you upon your submission of a properly certified voucher on or before the first date established in Article II of the contract. The remaining 50% of the agreed compensation will be paid to you within six months from the date of the first payment.

Performance of a cost audit of your expenditures has been eliminated through this lump sum payment for your research services. It is believed that this will save you considerable time and trouble in detailing your expenditures on cost reimbursement vouchers.

In order to assist you in preparing an appropriate voucher there is enclosed an instruction sheet containing numbered instructions corresponding with numbers appearing on a specimen copy of the voucher form. Vouchers should be submitted to the Oak Ridge Operations office in one original (white) and four copies (yellow) addressed as shown in Article IV of the contract. It is assumed that you will give your business office the benefit of these instructions.

Your attention is called to the reporting requirements outlined in Appendix "C" to the contract, especially to Item No. 2 requiring the immediate submission of a 200 word summary statement describing the purpose and scope of your project.

For your information and guidance in purchasing isotopes through the Commission, in accordance with the provisions of Article VII, there is enclosed a copy of the latest radioisotope catalog, together with a set of application forms, which you will use in making purchases of isotopes.

Your particular attention is invited to Appendix "B", Section 12 - Fellowships.

It is believed that the remaining portions of the contract are self-explanatory, however, if you have any questions concerning the application or interpretation of any of the contract provisions I will be glad to furnish you with additional information.

Very truly yours,

for C. Vanden Bulck
Assistant Manager
Oak Ridge Operations

Enclosures:
Contract (in quad.)
Vouchers & Instr. Sheets
Isotope Catalog & Applic. form w/instr. sheet

Nicholson:jn

UNITED STATES
ATOMIC ENERGY COMMISSION

LUMP SUM
RESEARCH CONTRACT

Contract No. AT-(40-1)-1324

THIS CONTRACT, entered into this 29th day of June, 19 51, by the UNITED STATES OF AMERICA (hereinafter called the "Government"), acting through the UNITED STATES ATOMIC ENERGY COMMISSION (hereinafter called the "Commission") and NORTH CAROLINA STATE COLLEGE (hereinafter called the "Contractor"):

ARTICLE I - PURPOSE AND SCOPE

1. The Commission, in furtherance of its policy of assisting and fostering private research, desires to support the Contractor's fundamental research in the field of atomic energy.

2. The work shall consist of a study of the effect of the composition of the diet on lipide metabolism.

The plan of approach to the problem and the agreed upon program and budget for the project are described in Appendix "A", which is hereby made a part of this contract. The Contractor shall be guided by, but not bound to conform to the details of the budget described in Appendix "A".

3. The Contractor shall furnish all services, facilities, equipment, supplies and materials (except such services, equipment, supplies and materials as the Government has agreed to furnish herein) required for the performance of the research program described in Section 2 above.

4. The work will be carried out by the Contractor under the direction of Dr. S. B. Tove as Project Leader

ARTICLE II - TERM OF CONTRACT

The initial period of performance for the research project covered by this contract will commence on July 1, 1951 and will end on June 30, 1952.
It is recognized that completion of the research work under this contract may involve a period of several years and that the term of this contract may be extended by mutual agreement.

ARTICLE III

1. Consideration. In consideration of the performance of the research activities described in Article I, the Government shall pay to the Contractor the sum of Five Thousand Dollars ($5,000.00) for the initial period of performance.

2. Payment

a. On or before the date of commencement of work on the project described in Appendix "A", the Government shall pay to the Contractor, upon submission by the Contractor of a properly certified voucher, one-half the amount of the agreed consideration.

b. On or before the expiration of six months from the date of commencement of the project, the Government shall pay to the Contractor, upon submission by the Contractor of a properly certified voucher, the remaining one-half of the agreed consideration.

c. In the event that the term of the contract is extended, the Government shall pay to the Contractor, upon submission by the Contractor of properly certified vouchers, each six months in advance an amount equal to one-half the annual agreed consideration for the project as mutually agreed upon by the parties hereto.

3. Program and Budget for Subsequent Periods. At least three months before the end of the initial period of performance of the project, the Contractor will submit to the Commission a current statement of its expenditures for the project, an estimate of expenses to be incurred during the remainder of the period, and a proposed program and budget for the succeeding year, showing the proposed work to be financed by the Commission and the Contractor. The Contractor and the Commission shall then negotiate as to the amount to be paid by the Commission to the Contractor for the services to be performed during the ensuing period, taking into consideration any portion of payments theretofore made which will remain unexpended at the end of the initial period. The extended program, budget and the additional amount to be paid to the Contractor shall be incorporated into a formal modification to this contract.

ARTICLE IV - ADMINISTRATION OF CONTRACT BY COMMISSION

The Commission has assigned the responsibility for administering the technical and scientific aspects for the project to the Washington organizational unit set forth below, to be addressed as follows:

Biology Branch
Division of Biology and Medicine

U. S. Atomic Energy Commission
1901 Constitution Avenue, N. W.
Washington 25, D. C.

Responsibility for administering the business aspects of this contract, including contract negotiations, budget, payment, audit, etc., has been assigned by the Commission to:

Office of Research & Medicine
Oak Ridge Operations Office
U. S. Atomic Energy Commission
Post Office Box E
Oak Ridge, Tennessee

The Contractor may, as necessary, communicate directly with the appropriate office, as indicated above. The Contractor shall furnish information copies of communications, memoranda of telephone conversations, or other contacts to Oak Ridge Operations Office on all direct dealings with the Washington Office.

ARTICLE V - REPORTS, RECORDS AND INSPECTION

1. The Commission shall have the right to inspect in such manner and at such times as it deems appropriate all activities of the Contractor arising in the course of the work under this contract.

2. The Contractor shall make progress and other reports in such manner and at such times as specified in Appendix "C" which is attached hereto and hereby made a part of this contract. Progress reports shall include a list of personnel working on the project. Names appearing for the first time should be accompanied by a brief statement of the individual's background, training, and experience.

3. The Commission shall at all times be afforded access to the premises and to all technical records, correspondence, instructions, drawings, and memoranda of record value of the Contractor pertaining to said work.

ARTICLE VI - TITLE TO PROPERTY PURCHASED BY CONTRACTOR

In consideration of the Contractor's contribution to the research project described in Appendix "A" of this contract, title to all materials, tools, machinery, equipment and supplies, acquired from any source including the Government, or manufactured by the Contractor under this contract shall vest in the Contractor, except that title to items of property described in Section 2.c. of Appendix "A" shall vest in the Government.

ARTICLE VII - PURCHASE OF RADIOISOTOPES

The Contractor shall purchase, to the extent available in appropriate form, all radioisotopes, irradiation services and cyclotron time required in the performance of the work hereunder, through the Commission's Isotope Division, Post Office Box E, Oak Ridge, Tennessee.

ARTICLE VIII - GENERAL PROVISIONS

The provisions of Appendix "B", attached hereto, are hereby made a part of this contract.

ARTICLE IX - AUTHORIZATION

This contract is authorized by and has been executed under the Atomic Energy Act of 1946.

ARTICLE X - ALTERATIONS

The following alterations to this contract were made by mutual agreement of the parties prior to its execution:

1. In Appendix "B", the third sentence of Section 3 is hereby deleted.

2. In Appendix "B", Section 10 Eight-Hour Law is hereby deleted in its entirety.

IN WITNESS WHEREOF, the parties hereto have executed this contract the day and year first above written.

UNITED STATES OF AMERICA

BY: UNITED STATES ATOMIC ENERGY COMMISSION

BY: C. Vanden Bulck

C. Vanden Bulck, Assistant Manager, ORO
Contracting Officer

NORTH CAROLINA STATE COLLEGE
(Contractor)

WITNESSES:

R W Cummings
N C State College
(Address)
Raleigh, N.C

Del Colvard
N.C. State College
(Address)

BY: J Vann

TITLE: Controller & Business Manager

ACCEPTANCE BY PROJECT LEADER

I have read the foregoing Contract and the Appendices attached hereto and made a part hereof, and I agree to be bound by the provisions of this document.

Samuel B Lee
Project Leader

Contract No. AT-(40-1)-1324
(North Carolina State College)

APPENDIX "A"

TITLE I

July 1, 1951 - June 30, 1952

This Title I describes the project and budget agreed upon between the Commission and the Contractor for the initial period of performance.

1. PROJECT

a. Scope and Plan of Approach

The project will attempt to determine the effect of the composition of the diet on lipide metabolism. The general objectives are:

(1) to determine whether the rate of either synthesis or catabolism of fatty acids, cholesterol and phospholipides is affected by a deficiency of one or more of the following dietary essentials: linoleic acid, biotin, pantothenic acid, pyridoxine and choline.

(2) to determine the mechanism by which lipide metabolism is altered by a deficiency of the aforementioned factors, should any metabolic changes become apparent.

(3) to explore interrelationships between those dietary essentials which appear to be involved in lipide metabolism.

The general procedure to be followed in the project involves feeding to rats a purified diet deficient in one or more of the factors to be studied. When characteristic deficiency symptoms appear, the animals will be injected with C^{14} labeled acetate (carboxyl labeled). After a suitable period, to be determined by pilot investigations, the rats will be sacrificed and the liver and carcass fat removed by solvent extraction. The phospholipides will be separated by acetone precipitation and the remaining fat saponified. Cholesterol

will be separated by digitonin precipitation, and the unsaturated fatty acids removed by bromination. The saturated fatty acids will be separated by chromatography. The amount of C^{14} in each of the isolated compounds will be counted. This will give a measure of the rate of synthesis of these metabolites. In addition the rate of synthesis of fatty acids, phospholipides and cholesterol will be studied in vitro. Tissue slices and washed tissue homogenates will be used in these studies. Rate of catabolism will be studied by the addition of suitable radioactive metabolites to tissue preparations such as described.

b. Materials, equipment and facilities

The Contractor will make available all necessary laboratory and work space, and equipment and facilities on hand, including the Warburg Barcroft respiration apparatus, automatic fraction collector, animal cages, radioactivity assay equipment, and analytical instruments.

c. Scientific Personnel

In addition to the Project Leader the key personnel working on the project will include F. W. Sherwood, F. H. Smith, and N. Underwood, and the following as advisers: G. H. Wise, H. L. Lucas, and C. K. Beck.

2. BUDGET - Period: July 1, 1951 - June 30, 1952

a. The Contractor will furnish as its contribution to the project:

(1) Salaries of staff members, including the Project Leader, and other personnel engaged in the work except as provided in b (1) below.

(2) Use of laboratory and work space and materials, equipment and facilities as described in 1. b. above, and such equipment and materials as are needed for the project in addition to the Government's contribution under b. below.

(3) All clerical, administrative and overhead costs.

b. The Government will pay to the Contractor the sum of Five Thousand Dollars ($5,000.00) to cover the Contractor's other expenses, estimated as follows, in the performance of the work under this contract:

(1)	Salaries	
	One graduate assistant	$1,500.00
(2)	Animals	200.00
(3)	Rations	550.00
(4)	Refrigerated centrifuge	2,000.00
(5)	Isotopes	550.00
(6)	Supplies	200.00
	Total	$5,000.00

c. Items of property procured or manufactured by the Contractor under this contract, title to which will vest in the Government (see Article VI): None.

APPENDIX "B"

GENERAL PROVISIONS

1. Patents

a. Whenever any patentable invention or discovery is made or conceived by the Contractor or its employees in the course of any of the work under this contract, the Contractor shall furnish the Commission with complete information thereon; and the Commission shall have the sole power to determine whether or not and where a patent application shall be filed, and to determine the disposition of the title to and rights under any application or patent that may result. The judgement of the Commission on these matters shall be accepted as final; and the Contractor, for itself and for its employees, agrees that the inventor or inventors will execute all documents and do all things necessary or proper to carry out the judgment of the Commission.

b. No claim for pecuniary award under the provisions of the Atomic Energy Act of 1946 shall be asserted by the Contractor or its employees with respect to any invention or discovery made or conceived in the course of any of the work under this contract.

c. Except as otherwise authorized in writing by the Commission, the Contractor will obtain patent agreements to effectuate the purposes of paragraphs a. and b. of this Article from all persons who perform any part of the work under this contract, except clerical and manual labor personnel who will not have access to technical data.

d. Except as otherwise authorized in writing by the Commission, the Contractor will insert in all subcontracts provisions making paragraphs a., b., and c. of this Article applicable to the subcontractor and its employees.

2. Publications. The Contractor shall have full freedom of publication of the results of the research under this contract and the Contractor is urged to disseminate the results of the work through customary scientific publication channels, except that "restricted data" as defined in the Atomic Energy Act of 1946 shall be governed by the provisions of Paragraph 3 of this Appendix "B". All publications shall include a reference that the results were developed under a Commission sponsored project.

3. Disclosure of Information.

a. It is understood that the work under this contract will not involve restricted data and the Contractor will perform such work as unclassified work. However, if in the course of such work any discoveries are made or any data used or developed that constitute restricted data, the Contractor shall promptly inform the Commission and shall classify and safeguard all discoveries and data in accordance with the requirements of the Commission. ~~It is understood that the person directing research work under this contract shall have been cleared by the Commission for access to restricted data.~~ The Contractor agrees that it will not permit any individual to have access to restricted data until the Federal Bureau of Investigation shall have made an investigation and report to the Commission of the character, associations and loyalty of such individual and the Commission shall have determined that permitting such person to have access to restricted data will not endanger the common defense or security. If doubt exists as to whether any discovery or data developed constitute restricted data, prior to the release of these data and before permitting any individual who has not received clearance from the Commission to have access to such data, the Contractor shall seek guidance from the Commission. Furthermore, the Commission reserves the right to require the classification of work whenever in its opinion restricted data are involved.

b. The continuation by the Contractor of work found to involve restricted data will be subject to mutual agreement of the Commission and the Contractor and shall be covered by a modification of this agreement. The phrase "restricted data" as defined in the Atomic Energy Act of 1946 and employed in this section shall mean "all data concerning the manufacture or utilization of atomic weapons, the production of fissionable material, or the use of fissionable material in the production of power, but shall not include any data which the Commission from time to time determines may be published without adversely affecting the common defense and security".

4. Disputes. Except as otherwise specifically provided in this contract, all disputes which may arise under this contract and which are not disposed of by mutual agreement shall be decided by a representative of the Commission duly authorized to supervise and administer performance under this contract, who shall reduce his decision to writing and cause a copy thereof to be mailed to the Contractor; said decision shall be final and conclusive, subject to the provisions of the sentence next following. Within thirty (30) days from the date of such mailing, the Contractor may appeal in writing to the Commission, whose written decision thereon, or that of its duly authorized representative, representatives, or board, not including the representative mentioned in the preceding sentence, shall be final and conclusive. Pending decision of a dispute hereunder, the Contractor shall proceed with the performance of its undertakings under this contract.

5. Safety and Accident Prevention - Inspections. The Contractor will comply with health and safety regulations of the Commission required for work of this nature, and permit the Commission and its designees to inspect the work conducted under this agreement.

6. Officials not to Benefit. No member of or Delegate to Congress, or Resident Commissioner shall be admitted to any share or part of this contract or to any benefit that may arise therefrom, but this provision shall not be construed to extend to this contract if made with a corporation for its general benefit.

7. Anti-Discrimination. The Contractor, in performing the work required by this contract, shall not discriminate against any employee or applicant for employment because of race, creed, color, or national origin.

8. Convict Labor. The Contractor shall not, in the performance of this contract, employ any person undergoing sentence or imprisonment at hard labor.

9. Termination.

a. The Commission may at any time upon 120 days written notice terminate this contract in whole or in part.

b. In the event of termination pursuant to subsection a., the Contractor will be reimbursed for the cost of the contract work already performed, together with reasonable costs of termination less the amount of all payments theretofore made. If the total payments theretofore made to the Contractor exceed the amount to which it is entitled hereunder, the Contractor shall promptly remit the amount of any such excess to the Government.

10. Eight-Hour Law. No laborer or mechanic doing any part of the work contemplated by this contract in the employ of the Contractor or any subcontractor contracting for any part of said work contemplated, shall be required or permitted to work more than eight (8) hours in any one calendar day upon such work at the site thereof, except upon the condition that compensation is paid to such laborer or mechanic in accordance with the provisions of this Article. The wages of every laborer and mechanic employed by the Contractor or any subcontractor engaged in the performance of this contract shall be computed on a basic day rate of eight (8) hours per day and work in excess of eight (8) hours per day is permitted only upon the condition that every such laborer and mechanic shall be compensated for

all hours worked in excess of eight (8) hours per day at not less than one and one-half (1½) times the basic rate of pay. For each violation of the requirements of this Article a penalty of Five Dollars ($5.00) shall be imposed upon the Contractor for each laborer or mechanic for every calendar day in which such employee is required or permitted to labor more than eight (8) hours upon said work without receiving compensation computed in accordance with this Article, and all penalties thus imposed shall be withheld for the use and benefit of the Government; provided, that this stipulation shall be subject in all respects to the exceptions and provisions of U. S. Code, Title 40, Sections 321, 324, 325, and 326, relating to hours of labor, as modified by the provisions of Section 303 of Public Act No. 781, 76th Congress, approved September 9, 1940, relating to compensation for overtime.

11. Definitions. As used in this contract the terms "United States Atomic Energy Commission", "Atomic Energy Commission" and "Commission" shall mean the United States Atomic Energy Commission or its duly authorized representative or representatives.

12. Fellowships. It is understood by the Contractor that none of the funds supplied by the Commission under this contract shall be used in any way to pay the stipend of any appointment for which commensurate services are not rendered under this contract; nor shall any of the funds be used to confer a fellowship, or to pay any part of the stipend of a fellowship, of any kind.

APPENDIX "C"

DISTRIBUTION AND SCHEDULING OF REPORTS
FOR DIRECT AEC RESEARCH CONTRACTS

	Date	Copies and Distribution	Remarks
CONTRACTOR REPORTS			
1. Progress	On one of Following: March 15 June 15 Sept. 15 Dec. 15	(2) Appropriate Washington Division (See note) (2) Oak Ridge Operations Office (See note)	To be received on date listed which is nearest to end of nine month period from effective date of contract and annually thereafter on the same date
2. Summary 200 words on scope and purpose	1. On completion of contract negotiation 2. With progress reports	Prepared as a part of contract negotiations (2) Same as Progress Reports	1. Distribution by Oak Ridge Operations Office with Contract copies 2. Revised Summary to be included as part of Progress Report
3. Manuscripts	As available	(1) Patent Branch, Washington (1) Technical Library, Washington (1) Appropriate Washington Division (1) Oak Ridge Operations Office	
4. Reprints	As available	(2) Appropriate Washington Division (2) Technical Information Branch, Washington (1) Oak Ridge Operations Office	
5. Complete Scientific Report	On Contract Termination	(1) Same (1) as (1) for (1) manuscripts	Manuscripts prepared for publication may in some cases take the place of this report
6. Brief Reports	As desired by investigator	(1) Appropriate Washington Office (1) Oak Ridge Operations Office	Covering significant results or developments.

NOTE: Full Addresses as follows:

Washington Offices:	Oak Ridge Operations Office
Atomic Energy Commission (Add name of Division or Branch) 1901 Constitution Avenue, N. W. Washington, D. C.	Atomic Energy Commission Office of Research and Medicine Post Office Box E Oak Ridge, Tennessee

The appropriate Washington Divisions are:

Division of Biology and Medicine - for contracts in Biology and Medicine.

Division of Research - for contracts in physical research.

AEC, Oak Ridge, Tenn.-1-24-50—2000-A5369

File: AEC, Copper

North Carolina State College of Agriculture and Engineering
of the
University of North Carolina
Raleigh

PHYSICS DEPARTMENT

October 19, 1951

Professor George H. Wise
304 Polk
Campus

Dear Professor Wise:

I have observed the second rehearsal of Dr. Tove's experiment involving radioactive copper. This cold run went very much better than the first one. There are two recommendations which I make before final approval; namely; (1) the general principle that no more radioactivity be kept out should be adhered to more carefully, (2) a Lucite or similar shield should be mounted on the syringes used for injection to provide protection to the hands of the operators.

Sincerely yours,

Newton Underwood

Newton Underwood
Radiological Safety Officer

NU:mm

CC: Dr. Samuel B. Tove

copy

Contract No. AT-(40-1)-1324
(North Carolina State College)

APPENDIX "A"

TITLE I

July 1, 1951 - June 30, 1952

This Title I describes the project and budget agreed upon between the Commission and the Contractor for the initial period of performance.

1. PROJECT

a. Scope and Plan of Approach

The project will attempt to determine the effect of the composition of the diet on lipide metabolism. The general objectives are:

(1) to determine whether the rate of either synthesis or catabolism of fatty acids, cholesterol and phospholipides is affected by a deficiency of one or more of the following dietary essentials: linoleic acid, biotin, pantothenic acid, pyridoxine and choline.

(2) to determine the mechanism by which lipide metabolism is altered by a deficiency of the aforementioned factors, should any metabolic changes become apparent.

(3) to explore interrelationships between those dietary essentials which appear to be involved in lipide metabolism.

The general procedure to be followed in the project involves feeding to rats a purified diet deficient in one or more of the factors to be studied. When characteristic deficiency symptoms appear, the animals will be injected with C^{14} labeled acetate (carboxyl labeled). After a suitable period, to be determined by pilot investigations, the rats will be sacrificed and the liver and carcass fat removed by solvent extraction. The phospholipides will be separated by acetone precipitation and the remaining fat saponified. Cholesterol

will be separated by digitonin precipitation, and the unsaturated fatty acids removed by bromination. The saturated fatty acids will be separated by chromatography. The amount of C^{14} in each of the isolated compounds will be counted. This will give a measure of the rate of synthesis of these metabolites. In addition the rate of synthesis of fatty acids, phospholipides and cholesterol will be studied in vitro. Tissue slices and washed tissue homogenates will be used in these studies. Rate of catabolism will be studied by the addition of suitable radioactive metabolites to tissue preparations such as described.

b. Materials, equipment and facilities

The Contractor will make available all necessary laboratory and work space, and equipment and facilities on hand, including the Warburg Barcroft respiration apparatus, automatic fraction collector, animal cages, radioactivity assay equipment, and analytical instruments.

c. Scientific Personnel

In addition to the Project Leader the key personnel working on the project will include F. W. Sherwood, F. H. Smith, and N. Underwood, and the following as advisers: G. H. Wise, H. L. Lucas, and C. K. Beck.

2. BUDGET - Period: July 1, 1951 - June 30, 1952

a. The Contractor will furnish as its contribution to the project:

(1) Salaries of staff members, including the Project Leader, and other personnel engaged in the work except as provided in b (1) below.

(2) Use of laboratory and work space and materials, equipment and facilities as described in 1. b. above, and such equipment and materials as are needed for the project in addition to the Government's contribution under b. below.

(3) All clerical, administrative and overhead costs.

b. The Government will pay to the Contractor the sum of Five Thousand Dollars ($5,000.00) to cover the contractor's other expenses, estimated as follows, in the performance of the work under this contract:

(1)	Salaries One graduate assistant	$1,500.00
(2)	Animals	200.00
(3)	Rations	550.00
(4)	Refrigerated centrifuge	2,000.00
(5)	Isotopes	550.00
(6)	Supplies	200.00
	Total	$5,000.00

c. Items of property procured or manufactured by the Contractor under this contract, title to which will vest in the Government (see Article VI): None.

UNITED STATES
ATOMIC ENERGY COMMISSION

RM:AMC

Oak Ridge, Tennessee

February 8, 1952

Chancellor J. W. Harrelson
North Carolina State College
Raleigh, North Carolina

Subject: RENEWAL OF CONTRACTS NO. AT-(40-1)-264, AT-(40-1)-265, AT-(40-1)-266, AT-(40-1)-1032, AT-(40-1)-1314, AT-(40-1)-1319, AT-(40-1)-1320, AND AT-(40-1)-1324

Dear Chancellor Harrelson:

We note that the subject contracts are due to expire this fiscal year, and suggest that if you plan to request renewal, your proposal be forwarded as soon as possible. This will permit the proper evaluation of research proposals submitted to the Atomic Energy Commission for consideration, and will avoid a rush toward the end of the fiscal year.

The following instructions may be helpful in preparing your proposal for consideration by the Commission:

1. A current statement of the expenditures incurred under the contract and an estimate of the obligations to be incurred during the remainder of the contract period should be submitted, together with the proposed program and budget for the succeeding year.

2. The progress report which is required at the end of nine months from the effective date of the contract may be submitted with the request for renewal, if you desire.

3. When your proposal is prepared, the following distribution should be made:

For contracts in the Physical Sciences:

1 original and 1 carbon copy to: U.S. Atomic Energy Commission
Division of Research
1901 Constitution Avenue, N.W.
Washington 25, D.C.

2 carbon copies to: U.S. Atomic Energy Commission
Office of Research and Medicine
P.O. Box E
Oak Ridge, Tennessee

S. B. Jones

For contracts in Biology and Medicine:

1 original and 1 carbon copy to: U.S. Atomic Energy Commission
Division of Biology and Medicine
1901 Constitution Avenue, N.W.
Washington 25, D.C.

2 carbon copies to: U.S. Atomic Energy Commission
Office of Research and Medicine
P. O. Box E
Oak Ridge, Tennessee

In the event you wish to discuss this matter, please do not hesitate to call upon us.

Very truly yours,

Kenneth Kasschau
Acting Director
Office of Research and Medicine

Corley:vh

CC: Dr. N.S. Hall, N.C. St. College
Dr. Walton C. Gregory, N.C. St. College
Dr. C. D. Van Cleave, N.C. St. College
Dr. Clifford Beck, N.C. St. College
Dr. D. S. Grosch, N.C. St. College
Dr. Kenneth O. Beatty, Jr., N.C. St. College
Dr. Frederick P. Pile, N.C. St. College
Dr. S. B. Tove, N.C. St. College

Form AEC-313
(Rev. March 1951)
B. B. No. 38-R027.1.

APPLICATION FOR RADIOISOTOPE PROCUREMENT

FOLLOW ATTACHED INSTRUCTIONS

LEAVE BLANK

TO: U. S. ATOMIC ENERGY COMMISSION, POST OFFICE BOX E, OAK RIDGE, TENNESSEE; ATTENTION: ISOTOPES DIVISION

1. NAME AND ADDRESS OF APPLICANT (*Institution, Firm, etc. Follow Instruction No. 2A*)
North Carolina State College
Raleigh, North Carolina

2. DEPARTMENT TO USE ISOTOPE (*Follow Instruction No. 2B*)
Animal Industry

3. NAME AND ADDRESS OF INDIVIDUAL USER (*Follow Instruction No. 3A*)
Dr. S. B. Tove, Department of Animal Industry, North Carolina State College, Raleigh, North Carolina

4. EXPERIENCE OF THE USER (*Follow Instruction No. 4B*)
Previous experiment with Cu^{64} on rabbits similar to the one proposed experiment. Participant in 18th class of ORINS Isotope technique course.

RADIOISOTOPE REQUESTED (*Follow Instruction No. 4*)

5. ISOTOPE (*Element and mass number*)	6. CHEMICAL FORM	7. QUANTITY (*Millicuries or irradiated units*)	8. OTHER SPECIFICATIONS
Cu^{64}	Copper	One irradiated unit (100 mc)	None

9. ITEM NO. (*If any*) IN U. S. AEC CATALOG
29

10. NAME AND ADDRESS OF SUPPLIER, IF KNOWN
Oak Ridge National Laboratory, Oak Ridge, Tennessee

STATEMENT OF USE (*Follow Instruction No. 5*)

11. STATE PROPOSED USE OF RADIOMATERIAL AND GENERAL PLAN OF INVESTIGATION

Upon arrival the radiocopper will be disolved in acid and diluted using a magnetic stirrer. Approximately 400 ug of copper (=50 uc) will be administered to each pig by either injection or feeding. Six pigs housed in the nutrition barn will be used. Periodic blood samples from each of the animals will be taken and counted until the radioactivity is no longer detectable. Fecal and urine samples will also be assayed for radioactivity, after wet ashing.

12. WILL THE RADIOISOTOPE BE USED IN HUMAN BEINGS? (*Follow Instruction No. 6A*) No — CIRCLE YOUR ANSWER — YES — (NO)

13. A. HUMAN DOSAGE (*In millicuries per patient*)	B. NUMBER OF DOSES (*Per patient*)	C. NUMBER AND TYPE OF PATIENTS	D. COMPOUND ADMINISTERED	E. SAMPLE TO BE TAKEN FOR MEASUREMENT

14. APPROVAL OF THE USER'S LOCAL ISOTOPE COMMITTEE (*Follow Instruction No. 6B*)
"THE LOCAL ISOTOPE COMMITTEE APPROVES THE HUMAN USE AS INDICATED IN ITEMS 11-13."

(Signature of Chairman, Local Isotope Committee)

15. WILL THE RADIOISOTOPE BE USED IN LOWER ANIMALS? Yes — CIRCLE YOUR ANSWER — (YES) — NO

16. IS A COMPLETED FORM AEC-313A A PART OF THIS APPLICATION? (*Follow Instruction No. 7*) — CIRCLE YOUR ANSWER — (YES) — NO

17. MAY THE ISOTOPES DIVISION RELEASE GENERAL INFORMATION REGARDING MATERIAL USED AND PURPOSE?
(*If your answer is "No," please state your reason here*)
CIRCLE YOUR ANSWER — YES — NO

READ THE TERMS AND CONDITIONS ON THE BACK OF THIS SHEET AND SIGN THE CERTIFICATE THAT FOLLOWS—AN UNSIGNED APPLICATION CANNOT BE CONSIDERED

16—57264-4

TERMS AND CONDITIONS

In consideration of the issuance of an authorization from the Commission to enable the applicant to procure or obtain the radioisotopes or irradiation service requested hereon, the applicant agrees that:

1. Radioisotopes purchased or acquired from the Commission or a distributor are shipped f. o. b. the laboratory, plant, facility, or Commission office handling the transaction, at prices and service fees as fixed by the Commission, and title to said materials, if same are not already owned by the applicant, shall pass to the applicant when the materials are delivered to the carrier. When shipment of the materials requires the use of returnable Government-owned containers, title to such containers shall remain in the Government and a deposit to insure return of the containers will be made if required. The applicant will keep the containers in good condition, will not use them for any materials other than the materials shipped therein, and will return them to point of shipment, transportation prepaid, within 21 days of date of shipment.

2. Neither the Government, the Commission, nor any distributor will be responsible for:

   (*a*) any damage to, destruction to, loss of, or changes in physical or chemical properties of materials of any kind accepted for a service irradiation, either as a result of, or in the process of, the irradiation or while said materials are in the possession of the Commission or a distributor;

   (*b*) any injury to persons or other living things or for damage to property caused by handling, shipment, use (including use based on any statement of quality or quantity), storage, transfer, disposal, or reshipment of, or other act or failure to act in connection with any materials purchased or acquired from the Commission or a distributor, or procured from any source upon the Commission's approval, it being expressly agreed that, as between the Commission, the supplying distributor, and the applicant, the applicant assumes complete responsibility and liability for any such injury or damage occurring: Provided, however, That if such injury or damage is caused solely by the negligent packing of the Commission or a distributor this assumption of liability shall not apply.

3. Neither the Government, the Commission, nor any distributor makes any warranty or other representation that (*a*) materials accepted for a service irradiation will not be destroyed, damaged, or otherwise altered in physical or chemical properties in the process of irradiation, and (*b*) radioisotopes (1) will not result in injury or damage when used for the purposes approved by the Commission, (2) will accomplish the results for which they are requested and approved by the Commission, (3) are safe for any other use, or (4) are of a particular quality or quantity. When procuring radioisotopes from the Commission or a distributor the applicant agrees to report promptly whether the amount received represents the amount paid for, in order that discrepancies may be adjusted.

4. Neither the Government, the Commission, nor any distributor shall be responsible, irrespective of cause, for the failure of the Commission, and distributor, or other transferor to (*a*) deliver radioisotopes at specified times, or (*b*) deliver radioisotopes of specified quality.

5. When materials supplied for a service irradiation are:

   (*a*) from an applicant not authorized to possess or use radioisotopes, the Commission or the distributor shall have the right to retain possession and control of the irradiated materials throughout the period of measurable activity of such materials, and unless otherwise stated in the request for service irradiation, may dispose of such materials in accordance with the usual Commission or distributor disposal procedures for radioactive materials;

   (*b*) to be tested or analyzed and retained by the Commission or a distributor, such materials may, unless otherwise stated in the request for service irradiation, be disposed of in accordance with the usual Commission or distributor disposal procedures for radioactive materials.

   It is expressly agreed that if any irradiated materials covered by (*a*) or (*b*) above must be retained by the Commission or a distributor in order to protect health and minimize other hazards to life or property, the applicant will pay all storage and maintenance charges connected therewith, and if any irradiated materials belonging to the applicant are disposed of under the provisions of this paragraph, the applicant shall have no claim for the value or replacement of said materials.

6. The Commission shall have the right to publish and use any information or knowledge acquired as a result of the irradiation of materials furnished by the applicant, including results of tests and analyses made for the applicant in connection with any such irradiated materials.

7. The right to revoke or cancel, with or without cause, arrangements for or agreements for the purchase or acquisition of any radioisotopes from a distributor, including arrangements or agreements for service irradiations, is reserved to the Commission. In the event the Commission revokes or cancels any arrangement or agreement for a service irradiation, the Government, the Commission, and the distributor shall be discharged of all obligations thereunder by return to the applicant of an amount of nonirradiated material of like kind, quality, and quantity as the material accepted for irradiation.

8. Title to and possession of all radioisotopes purchased or acquired from the Commission or from a distributor, or from any source on the authorization or approval of the Commission, remain subject to the Commission's statutory *right to recall*. Title to any materials recalled by the Commission shall vest in the Commission with the exercise of this right, and the Commission may enter and take possession of said materials any time after notice is given that the materials are being recalled: Provided, That if requested, the applicant, at his expense, will make shipment of the recalled materials to a destination designated by the Commission.

9. The applicant agrees to indemnify the Government, the Commission, their officers, agents, contractors, distributors, servants, and employees against liability, including costs and expenses incurred, for infringement of any Letters Patent occurring in the course of any service irradiation, test, or analysis performed for the applicant by the Commission or its distributors, or occurring in the utilization by the applicant of any radioisotopes or irradiated materials.

10. The applicant will furnish to the Isotopes Division six copies of each article published on the results of his investigations using radioisotopes or irradiation services, or will upon request furnish to the Isotopes Division a report of the results of his investigations.

11. Any radioisotopes received as a consequence of this application will be dealt with in accordance with all instructions, recommendations, or standards issued by the Commission for the safe use, handling, or disposal of radioactive materials.

12. All purchase orders and agreements for procuring radioisotopes are subject to the terms and conditions hereof and any contrary conditions of sale or transfer contained in such purchase orders or agreements will not apply.

CERTIFICATE

The applicant and any official executing this application in behalf of the applicant certify that the information stated herein is true and correct, that this application is made under and in conformity with Code of Federal Regulations, Title 10, Atomic Energy, Part 30, Radioisotope Distribution, and agree that this application and any materials procured pursuant thereto are subject to the terms and conditions on this page.

(Date)

(Signature of Applicant or Certifying Official)

(Title)

WARNING

18 U. S. C., Sec. 1001; act of June 25, 1948; 62 Stat. 749; makes it a criminal offense to make a willfully false statement or representation to any department or agency of the United States as to any matter within its jurisdiction.

16—57264-4

February 20, 1952 A.E.C. File

TO THE FACULTY OF STATE COLLEGE:

Under the terms of an agreement reached between North Carolina State College and representatives of the Atomic Energy Commission, State College has agreed to accept the responsibility for distribution of radioisotopes to qualified staff members who have adequate facilities, and for requiring that accepted safety precautions in the use of radioisotopes be observed. The commission, in turn, has agreed to remove all restrictions in prior approval proeedures, etc., on the purchase of isotopes by State College faculty, except on those intended for use in experiments involving human beings and those involving widespread field or soil application.

In order for State College to discharge its responsibility under this agreement, I have appointed a Radioisotope Committee, consisting of:

D. B. Anderson
C. G. Brennecke
N. S. Hall
F. H. Smith
Newton Underwood
Clifford K. Beck, Chairman

I have asked this Committee to devise procedures by which our obligations can be fulfilled. The bulletin attached hereto defines these procedures, the responsibility and function of the Radioisotope Committee, the procedures by which radioisotopes may now be procured by faculty members of State College, and the regulations governing use of these isotopes on our campus.

Your observance of these procedures is requested and your cooperation in this matter is appreciated.

Sincerely yours,

(Signed) J. W. Harrelson
Chancellor

PROCEDURE FOR OBTAINING RADIOISOTOPES

AND

REGULATIONS REGARDING USAGE OF RADIOISOTOPES

AT

NORTH CAROLINA STATE COLLEGE

Issued By

RADIOISOTOPE COMMITTEE

D. B. Anderson
C. G. Brennecke
N. S. Hall
F. H. Smith
Newton Underwood
Clifford K. Beck, Chairman

August 6, 1951

PROCEDURE FOR OBTAINING RADIOISOTOPES
AND
REGULATIONS REGARDING USAGE OF RADIOISOTOPES
AT
NORTH CAROLINA STATE COLLEGE

Introduction

For several years, the Atomic Energy Commission has made available to qualified individuals a variety of radioisotope for research purposes. Numerous shipments have been received by State College Staff Members. In the past, radioisotopes have been obtained by a three-step procedure:

1. The individual requested authorization of the A. E. C. to purchase a specified radioisotope for a specified purpose.

2. The A. E. C. ascertained, by information furnished by the requestor on standard forms, that the person requesting the radioisotope was competent to handle the desired radioisotope, then issued permission for the isotope to be purchased.

3. A purchase order, accompanied by the A. E. C. approved permit, was issued for the procurement of the radioisotope.

By this procedure, the A. E. C. was responsible for ascertaining that the person requesting the radioisotope was competent to handle it in the proposed experiments without endangering this own safety or that of others, and also was responsible for approving or disapproving the proposed experiments from the standpoint of their potential hazards to the safety of personnel, to area contamination, to good public relations in the community, etc.

The A. E. C. has devised a new system by which the responsibilities described above have been transferred to State College when radioisotopes are used by college staff members.

Under the new system, when an individual desires to procure a radioisotope, the college itself must ascertain:

1. That the individual is competent to handle safely the radioisotope desired.

2. That adequate equipment and facilities are available for safe conduct of the proposed experiments.

3. That the proposed experiments will not involve excessive potential hazard to community welfare, to wide area contamination--whether harmful or not, and to good public relations.

This general agreement does not cover two categories of uses; (1) Human experimentations, and (2) Experimentation involving widespread distribution of radioisotopes; for example, in fields or soils. For experiments of these types, each proposed usage must be separately approved by the A. E. C.

When the College has satisfied itself on these points, the desired radioisotope may be purchased directly, without outside approval.

Assignment of Responsibility

After extensive consultation with the A. E. C., a formal commitment by State College has been made to accept the responsibilities described above. A procedure has been established and a functioning committee appointed through which these responsibilities within the College can be carried out. The procedure has been formally approved by both Chancellor Harrelson and the A. E. C. representative, and the Radioisotope Committee and the Raeiological Safety Officer have been directed to function as described below.

Procedure for Procurement of Radioisotopes

The following procedure for procuring radioisotopes has been established:

1. Three copies of "Request for Radioisotopes," Form RIC-1 (sample attached hereto), completely filled out, are sent to the Chairman of the Radioisotope Committee, along with a completed Purchase Requisition for the desired materials.

(a) The original of the Request is returned to the requestor, with the action of the Radioisotope Committee indicated.
(b) The first carbon is placed in the Radioisotope Committee's files.
(c) The second carbon is sent to the Radiological Safety Officer (RSO).
(d) The purchase requisition, if approved, is sent directly to the Purchasing Department.

The Purchasing Department has been directed to make no procurement of radioisotopes unless prior approval of the Radioisotope Committee has been given.

RESPONSIBILITIES REGARDING USE OF RADIOISOTOPES

A. The Radioactive Isotope Committee

1. As indicated above, the Radioisotope Committee of State College must approve the proposed usage of any radioactive isotope before that isotope may be procured.

2. After a radioactive isotope is obtained, any proposed extension or alteration of its use beyond that previously approved must also be approved or disapproved by the Radioisotope Committee.

NOTE: The approval or disapproval of proposed isotope usage is based solely on the three criteria: Safety of personnel, area contamination, public good-will, and is in no way related to judgment of the scientific merit, feasibility or adequacy of the proposed experiments.

3. It is the responsibility of the Committee to require adherence to established practices and safe procedures in any manipulations involving radioisotopes on the campus. The committee shall demand the cessation of any radioisotope project in which unsafe practices are discovered, and the project may not continue until the Committee is satisfied with the safety practices adopted.

4. The Committee must receive periodic reports from the Radiological Safety Officer and must investigate promptly any radioisotope manipulations reported by the R.S.O. to be unsafe.

5. The Committee must maintain records of the matters considered and the decisions reached.

B. The Radiological Safety Officer (RSO)

1. Shall be a member of the Radioisotope Committee of the college.

2. Shall decide with the Committee what inspection, monitoring, spot checking and other investigation of the use of radioisotopes on the campus should be made, shall be responsible for making these investigations, and shall report at periodic intervals to the committee the results of such investigations.

3. May investigate the use and method of handling of any radioisotope on the campus at any time.

4. Must report at once to the committee, or to the chairman, if the committee is not in session, any unsafe conditions or practices observed in the use of isotopes on the campus.

5. May demand cessation of any project pending subsequent action of the committee, if grossly unsafe conditions or practices are observed.

6. Must keep adequate records on observations, inspections, and investigations of the use of radioisotopes on the campus.

7. Shall have reported to him as promptly as possible any accident or spill of radioisotopes on the campus.

C. Individual User of Radioisotopes

1. Must furnish information requested by the Committee and by the R.S.O., both at the time of original approval and at any subsequent time requested, regarding the proposed usage of isotopes and the procedures expected to be used. This information, at the time of original approval, shall include a complete "cold-run" demonstration of the anticipated procedures.

2. Must present for Radioisotope Committee approval any new or extended use of a radioisotope beyond that approved by the Committee when the isotope was purchased.

3. Must report as promptly as possible to the R. S. O. any accident involving radioactive isotopes or any inadvertent spill of radioisotope on the campus.

Appendix A, attached hereto, contains a partial list of general principles by which the Radioisotope Committee will be guided in approving or disapproving proposed uses of Radioisotopes.

REQUEST FOR RADIOISOTOPE

Form RIC-1

Date: _____

Name: _____

Date: _____

Location: Office: _____ Lab: _____

Phone: _____

Radioisotope Desired:

(a) Active element (name and mass) _____

(b) Chemical form _____

(c) Amount of radioactivity _____

(d) Types and energy of radiation _____

(e) Half life _____

Anticipated Usage:

(a) Objective of the experiments _____

_____

(b) Role of the radioisotope _____

_____

(c) Outline of Experimental Procedure

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

Experience in Experiments with Radioisotopes (Reference may be made to prior application, with date cited.)

(a) Previous experiments at State College _____

_____

(b) Radioisotope training _____

_____

Special Equipment Available (Reference may be made to prior application, with date cited.)

Hoods _____

Shielding _____

Remote manipulators _____

Survey Meters _____

Radioactivity measuring instruments _____

_____

Other _____

Report of Radiological Safety Officer

_____

_____

_____

Action of Radioisotope Committee

_____

_____

_____ Chairman

Radioisotope Committee

Date _____

Appendix A

August 3, 1952

Generally Accepted Techniques and Regulations Involving Radioisotopes

1. Manipulation of radioisotopes in open containers should be done in a hood.

2. Work in closed systems outside of a hood should be done with a slightly negative pressure in the apparatus. Where experimental procedure demands that the apparatus function at atmospheric pressure and it will not fit into a hood, it should be carefully sealed and the existence of an extra hazard should be recognized.

3. Any system that involves the possibility of an explosion (such as wet ashing with acid and perchlorate solution) must be operated in a hood equipped with safety glass.

4. Precautions should be taken against spillage. If spillage should occur, evacuate personnel and monitor them, post region of spill and notify R.S.O.

5. Label all radioactive containers and post signs indicating radioactive hazard. Label on containers should identify the chemical compounds, the type of activity, the date stored, and the quantity of radioactivity.

6. Experiments should be planned and executed so as to involve as limited an area in a building as possible. It is highly desirable that the work be confined to one room or part of a room except for the counting of specimens.

7. The amount of radioactivity handled at any one time should be kept to the minimum value necessary for the given procedure.

8. The disposal of radioactive waste is such a specialized problem that the individual case needs to be studies and a definite disposal plan worked out. Where long lived isotopes are involved, and it is economically feasible the isotope should be recovered for re-use.

UNITED STATES
ATOMIC ENERGY COMMISSION
WASHINGTON 25, D. C.

IN REPLY REFER TO:

BMB:HHP

APR 3 0 1952

Dr. S. B. Tove
North Carolina State College
Raleigh, North Carolina

Dear Dr. Tove:

Your request for renewal of your research contract with the change in subject and major line of study was presented to the research committee at a recent meeting. They expressed considerable interest in the main line of your investigation and the metabolism lipid substance using labeled sodium acetate. A slight change was made in the title of the project to indicate more clearly the reason for AEC support, and it now reads as follows: "A Study of the Effect of the Diet on Lipid Metabolism Using C-14."

The project was approved at approximately the amount requested, and it is hoped that you will be able to make some progress on this work during the coming year.

You may expect to hear from the Oak Ridge Operations Office regarding the negotiations of the contract within the near future.

Sincerely yours,

H H Plough

Harold H. Plough
Assistant Chief, Biology Branch
Division of Biology and Medicine

UNITED STATES
ATOMIC ENERGY COMMISSION

DC:JN

Oak Ridge, Tennessee
May 26, 1952

North Carolina State College
Raleigh, North Carolina

Attention: Dr. S. B. Tove

Subject: MODIFICATION NO. 1 TO CONTRACT NO. AT-(40-1)-1324

Gentlemen:

Enclosed, in triplicate, is Modification No. 1 to the contract numbered as shown in the subject line above, which incorporates in a revision to Appendix "A" the program and budget for the period beginning July 1, 1952 and ending June 30, 1953.

If the modification, as submitted, is satisfactory to you it is requested that you sign the three copies thereof in the space provided for the Project Leader and have the modification signed by the proper official of the College, returning the three copies to this office. After execution on behalf of the Commission, one duly executed copy and one conformed copy of the modification will be returned to you for your retention.

Very truly yours,

R. G. Humphries
Acting Director
Contract Division

Enclosure:
Mod. 1 (in trip.)

Nicholson:jn

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
PROJECT OUTLINE

Project No. AEC-5
Date
Submitted .....
Approved .....
Revised .....

1. Title **Effect of the composition of the diet on lipide metabolism.**

2. Objective(s)

a. To determine whether the rate of either synthesis or catabolism of fatty acids, cholesterol and phospholipides is affected by a deficiency of one or more of the following dietary essentials: linoleic acid, biotin, pantothenic acid, pyridoxine and choline.

b. To determine the mechanism by which lipide metabolism is altered by a deficiency of the aforementioned factors, should any metabolic changes become apparent.

c. To explore interrelationships between those dietary essentials which appear to be involved in lipide metabolism.

3. Reasons for undertaking Investigations* **The basic program of the Animal Nutrition Section of the Department of Animal Industry is to investigate fundamental problems relating to the nutrition and metabolism of animals with the ultimate aim of applying any information gained to livestock production. The proposed project is in keeping with this policy.**

There is little direct evidence concerning the exact metabolic pathways by which lipides are synthesized and degraded in animal tissues. This is largely due to the fact that these processes are intimately associated with a series of complex oxidative and phosphorylative systems. Inasmuch as it has not been possible to separate and study the individual metabolic reactions of these systems, the use of experimental procedures that proved to be valuable in elucidating the pathways of carbohydrate metabolism have not been applicable. It is well established that a vitamin deficiency results in a block of the metabolic pathway in which this nutrient is involved; although these blocks are often difficult to detect by the usual analytical techniques of the biochemist. The great selectivity and sensitivity inherent in the tracer technique overcomes many of these difficulties and, thus, the use of isotopic tracers becomes a virtual necessity in the study of lipide metabolism. Since all of the aforementioned vitamins have been implicated in lipide metabolism (see below), it is presumed that a deficiency of one or more of these factors would produce a metabolic block detectable by the sensitive tracer technique. If the foregoing hypothesis is valid, such an experimental approach should permit a more detailed study of lipide metabolism.

*Including economic justification

Project No. AEC-5

4. Previous work and present status of investigations in the field of this project:

Although twenty years have passed since Burr and Burr (J. Biol. Chem. 82:345, 1930) observed that a dermatitis which rats develop on a fat-free diet can be cured by small amounts of linoleic or arachidonic acids, the specific function of these compounds is unknown. It has been assumed, generally, that linoleic acid and arachidonic acid were concerned with the formation of phospholipides and that the latter compounds were important in the metabolism of fat. On the other hand, Smedley-Maclean and Munn (Biochem. J. 34:884, 1940) have shown that in an essential fatty acid deficiency there is no decrease in liver phospholipides and that muscle phospholipides increased when linoleic acid was decreased from one drop to one-half or one-fourth drop per rat per day.

It has been established that biotin can replace oleic acid for the growth of lactic acid bacteria (J. Biol. Chem. 169:761, 1947; 170:619, 1947; 172:531, 1948). Gavin and McHenry (J. Biol. Chem. 141:619, 1941) have shown that under certain conditions biotin can cause fatty livers and an increase in the amount of C_{18} fatty acids as well as unsaturated fatty acids. Thus, it would appear likely that biotin functions in the synthesis of fatty acids in animals as well as in bacteria. This hypothesis is to be tested.

Several workers have shown that pyridoxine is required for the complete cure of the syndrome described by Burr and Burr (J. Biol. Chem. 124:775, 1938; 132:41, 1940; 140:CIX, 1941). Although this vitamin is known to be involved in amino acid metabolism, apparently its role in fat metabolism has not been investigated.

(Continued on attached page)

5. Outline of Procedure:

The general procedure involves feeding to rats a purified diet deficient in one or more of the factors to be studied. When characteristic deficiency symptoms appear, the animals will be injected with C^{14} labeled acetate (carboxyl labeled). After a suitable period, to be determined by pilot investigations, the rats will be sacrificed and the liver and carcass fat removed by solvent extraction. The phospholipides will be separated by acetone precipitation and the remaining fat saponified. Cholesterol will be separated by digitonin precipitation, and the unsaturated fatty acids removed by bromination. The saturated fatty acids will be separated by chromatography following the procedure of Howard and Martin (Biochem. J. 46:532, 1950). The amount of C^{14} in each of the isolated compounds will be counted. This will give a measure of the rate of synthesis of these metabolites. In addition the rate of synthesis of fatty acids, phospholipides and cholesterol will be studied in vitro. Tissue slices and washed tissue homogenates will be used in these studies. Rate of catabolism will be studied by the addition of suitable radioactive metabolites to tissue preparations such as described.

The basal diet will consist of:

Sucrose	76
Vitamin-free casein	20
Salts	4
Cystine	0.2
Methyl linoleate	0.1 gm/day given by dropper.

(Continued on attached page.)

4. Previous work and present status continued:

Pantothenic acid has been shown to be part of coenzyme A, which is associated with the metabolism of acetate (J. Biol. Chem. 160:173, 1945; 167:869, 1947). Since it is probable that fatty acids are synthesized from acetate and broken down via acetate, one would expect a pantothenic acid deficiency to have a marked effect on fat metabolism. A recent paper (Fed. Proc. 10:226, 1951) suggests that pantothenic acid is required for the synthesis of cholesterol.

Since choline is part of the molecule of phospholipides and these latter compounds are concerned with fat metabolism, this vitamin should have an effect on the rate of formation and/or breakdown of fat. A choline deficiency would be useful in ascertaining whether any observed changes in fat metabolism are due to lack of synthesis of fatty acids or lack of phospholipide formation and reduced mobility of fat from one part of the body to another.

5. Outline of Procedure continued:

The following vitamins will be added in mg./100 gm. ration:

Thiamin	0.2	Biotin	0.01
Riboflavin	0.3	Folic acid	0.2
Pyridoxine	0.25	Menadione	0.5
Ca pantothenate	2.0	Inositol	10.0
Niacin	1.0	Vitamin B_{12}	0.005
Choline	100.0		

Vitamins A, D and E are to be given twice weekly in methyl laurate.

The following diets will be tested:

1. Basal
2. Basal less methyl linoleate
3. Basal less choline
4. Basal less pantothenic acid
5. Basal less pyridoxine
6. Basal less methyl linoleate and choline
7. Basal less methyl linoleate and pyridoxine
8. Egg white basal (egg white replacing casein, biotin injected)
9. Egg white basal less biotin
10. Egg white basal less biotin and methyl linoleate
11. Egg white basal less methyl linoleate

During the period between the injection of the radioacetate and death, the rats will be placed in metabolism cages in a hood. There will be no more than four animals in the hood at any one time. The maximum dose any rat will receive will be 50 uc of C^{14}. The hood has a forced draft of 1300 cu. ft. of air per minute and is vented directly through the roof of the building. Thus, if 200 uc or radiocarbon were expired by the rats within one minute, the concentration in the air leaving the hood would be 0.53×10^{-5} uc per cc. of air. This is approximately half the concentration of radiocarbon designated as safe for human respiration by the Atomic Energy Commission (AEC Isotopes Division Circular B-5).

All carcasses, excreta and contaminated matter will be disposed of by incineration in the presence of an excess of carbonaceous matter. Separation of C^{14} containing compounds will be carried out in a closed system. All of the components to be separated are very slightly volatile.

A pilot experiment will be conducted on thirty rats using diets 1, 4 and 8. The purpose of this pilot experiment will be to determine the turnover rate of fatty acids, cholesterol and phospholipides under the proposed experimental conditions and to serve as a basis for the efficient planning of future experiments.

6. Probable Duration of Project: Three years

7. Date of Initiation: July 1, 1951

8. Personnel:

Name	Department	Relation to Project
S. B. Tove	Animal Industry	Leader
F. W. Sherwood	Animal Industry	Co-leader
F. H. Smith	Animal Industry	Co-leader
N. Underwood	Physics	Cooperator
G. H. Wise	Animal Industry	Adviser
H. L. Lucas	Institute of Statistics	Adviser
C. K. Beck	Physics	Adviser

9. Coöperation:

a. Interdepartmental - Department of Animal Industry, Institute of Statistics and Physics Department

b. Other Agencies - United States Atomic Energy Commission

10. Financial Support:

a. Proposed Budget ..... to .....

Items	ALLOCATION OF FUNDS					
	Bankhead-Jones	Purnell	Adams	State	AEC Other	Total
1. Salaries						4100.00
S. B. Tove				2000.00		
F. W. Sherwood				200.00		
F. H. Smith				200.00		
G. H. Wise				200.00		
Graduate Assistant					1500.00	
2. Labor				500.00		500.00
3. Travel				25.00		25.00
4. Equipment & Supplies				200.00	3500.00	3700.00
5. All Other						
Total				3325.00	5000.00	8325.00

b. Proposed Future Budgets:

Year	Salaries	Total Expenditures	Estimated Income
1952-1953	$4,100.00	$7,825.00	None

11. General Remarks:

Project No. AEC-5

SIGNATURES OF APPROVAL

1. Approval of Project Leaders

Date September 25, 1952 ... Samuel B. Fox

Title Assist. Res. Prof.

Date ... J. H. Smith

Title Assoc. Res. Prof.

Date ...

Title ...

2. Approval of Heads of Departments or Coöperating Agencies

Date Sept 26, 1952 ... D. A. Coleman

Head, Animal Industry Dept.

Date Sept 30, 1952 ... C. Upchurch Cash

Head, Dept. of Physics

Date ...

Head, ...

3. Approval of Committee on Experiment Station Projects

Date ...

Chairman of Committee

4. Approval of Director

Date 10/8/52 ... R. W. Cummings

Director, North Carolina Agricultural Experiment Station

5. Approval of U. S. D. A.

Date ...

Chief, Office of Experiment Stations

Files of Animal Nutrition

REQUEST FOR RENEWAL OF CONTRACT

A. E. C. Contract: #AT-(40-1)-1324

Contractor: North Carolina State College

Title: A Study of the Effect of the Composition of Diet on Lipide Metabolism.

Status of Project:

A considerable part of the past year was spent in refining the isolation techniques so that they would be suitable for the small amounts of lipide materials involved. Consequently, at the present time there exists a backlog of about forty samples of liver fat, which are in the process of fractionation. The isolation procedures are lengthy, requiring about sixty hours to complete the fractionation of the lipides of a single liver sample.

Proposed Work:

In addition to completing the samples on hand, which will yield data on biotin and pantothenic acid deficiencies, the effects of choline, linoleic acid and pyridoxine deficiencies are to be studied as outlined in the original project.

It was not possible to obtain a graduate assistant with suitable qualifications to fulfill the appointment created by the contract. The funds allotted for the assistantship, therefore, were used to hire a laboratory technician on a part-time basis. In view of the lengthy fractionation procedures, it is requested that sufficient funds be provided to hire a full-time technician. Furthermore, at the present time, the probability of obtaining this type of assistance is better than that of obtaining a graduate student with suitable background.

The progress of the project could be further expedited through the use of another flow-gas counter on the campus. Hence, it is requested that funds be made available for the purchase of a windowless flow counter.

Expenditure of Funds
1951-1950

Budget of Available Funds:	
Salaries (Graduate Assistant)	$ 1,500.00
Equipment (Refrigerated Centrifuge)	2,000.00
Supplies (Animals, rations, isotopes, etc.)	1,500.00
	$ 5,000.00
Funds Spent as of March 1, 1952	
Salaries (part-time technician)	$ 939.80
Supplies	494.07
Equipment	- -
	$ 1,433.87
Funds Anticipated to be Spent by July 1, 1952	
Salaries (part-time technician)	$ 560.20
Equipment (Refrigerated Centrifuge)	2,000.00
Supplies	1,005.93
	$ 3,566.13

At present, there is one outstanding bid for the refrigerated centrifuge as provided in the 1951-52 contract, and as soon as this is received the instrument will be ordered. It is expected that the cost will be in the neighborhood of $2,400.00. If it is agreeable with the Atomic Energy Commission, the additional $400.00 required would be transferred from the supply fund.

Budget Proposed for 1952-1953

Salaries (full-time laboratory technician)	$ 3,200.00
Equipment (Windowless flow counter)	450.00
Animals	150.00
Rations	300.00
Isotopes	150.00
Supplies	250.00
	$ 4,500.00

Please return to Animal Nutrition

UNITED STATES
ATOMIC ENERGY COMMISSION

OR:AMC

Oak Ridge, Tennessee
April 6, 1953

Chancellor J. W. Harrelson
North Carolina State College
Raleigh, North Carolina

Subject: RENEWAL OF CONTRACTS NO. AT-(40-1)-264, AT-(40-1)-265, AT-(40-1)-1319, AT-(40-1)-1320, and AT-(40-1)-1324

Dear Sir:

We note that the subject contracts are due to expire this fiscal year, and suggest that if you plan to request renewal, your proposal be forwarded as soon as possible. This will permit the proper evaluation of research proposals submitted to the Atomic Energy Commission for consideration, and will avoid a rush toward the end of the fiscal year.

The following instructions may be helpful in preparing your proposal for consideration by the Commission:

1. A current statement of the expenditures incurred under the contract and an estimate of the obligations to be incurred during the remainder of the contract period should be submitted, together with the proposed program and budget for the succeeding year.

2. The proposal should contain a statement of acceptance, signed by an official of the institution.

3. The progress report which is required at the end of nine months from the effective date of the contract may be submitted with the request for renewal, if you desire.

4. When your proposal is prepared, the following distribution should be made:

For contracts in the Physical Sciences:

1 original and 1 carbon copy to: U. S. Atomic Energy Commission
Division of Research
1901 Constitution Ave., N. W.
Washington 25, D. C.

2 carbon copies to: U. S. Atomic Energy Commission
Research & Medicine Division
Post Office Box E
Oak Ridge, Tennessee

(GVR)

For contracts in Biology and Medicine:

1 original and 1 carbon copy to: U. S. Atomic Energy Commission
Division of Biology & Medicine
1901 Constitution Avenue, N.W.
Washington 25, D. C.

2 carbon copies to: U. S. Atomic Energy Commission
Research & Medicine Division
Post Office Box E
Oak Ridge, Tennessee

In the event you wish to discuss this matter, please do not hesitate to call upon us.

Very truly yours,

Kenneth Kasschau

Kenneth Kasschau
Director
Research and Medicine Division

Corley:smh

CC: Dr. N. S. Hall
Dr. W. C. Gregory
Dr. K. O. Beatty, Jr.
Dr. F. P. Pike
Dr. S. B. Tove

North Carolina State College of Agriculture and Engineering
of the
University of North Carolina
Raleigh

SCHOOL OF AGRICULTURE
RESEARCH EXTENSION
RESIDENT TEACHING

OFFICE OF DEAN AND DIRECTORS

April 22, 1953

COPY

U. S. Atomic Energy Commission
Division of Biology & Medicine
1901 Constitution Avenue, N. W.
Washington 25, D. C.

Gentlemen:

I am forwarding herewith the original and one carbon of a progress report under our Contract No. AT-(40-1)-1324 entitled "A Study of the Effect of the Composition of Diet on Lipide Metabolism Using C^{14}", and a request for renewal of this contract for the fiscal year 1953-54. This request has been prepared by our Department of Animal Industry and has the concurrence of the Director of Research and the Assistant Controller and Business Manager of the Institution. We would appreciate very much your favorable consideration of this request.

Sincerely yours,

R. W. Cummings
Director of Research.

Approved: _____
J. G. Vann, Assistant Controller
and Business Manager

North Carolina State College of Agriculture and Engineering
of the
University of North Carolina
Raleigh

SCHOOL OF AGRICULTURE
RESEARCH EXTENSION
RESIDENT TEACHING

OFFICE OF DEAN AND DIRECTORS

April 22, 1953

COPY

U. S. Atomic Energy Commission
Research and Medicine Division
Post Office Box E
Oak Ridge, Tennessee

Gentlemen:

I am enclosing copies of our progress report and our request for renewal of our Contract No. AT-(40-1)-1324 entitled "A Study of the Effect of Diet on Lipide Metabolism Using C^{14}". The original has been sent to the Division of Biology and Medicine, U. S. Atomic Energy Commission, Washington D. C. We sincerely hope that this request will receive favorable consideration.

Very truly yours,

R. W. Cummings
Director of Research.

File A.E.C.

UNITED STATES
ATOMIC ENERGY COMMISSION
WASHINGTON 25, D. C.

IN REPLY REFER TO:

BMB:PBP

May 6, 1953

Dr. R. W. Cummings, Director
Agriculture Experiment Station
North Carolina State College
Raleigh, North Carolina

Dear Dr. Cummings:

I want to take this occasion to tell you that I thoroughly enjoyed my visit to North Carolina State and the opportunity to see the fine work that is going on on the Atomic Energy Commission projects there. I hope you will express my appreciation to the various members of the staff who were so generous with their time in showing me around the college and the work they are doing.

Sincerely yours,

Paul B Pearson

Paul B. Pearson
Chief, Biology Branch
Division of Biology and Medicine

UNITED STATES
ATOMIC ENERGY COMMISSION
WASHINGTON 25, D. C.

IN REPLY REFER TO:

BMB:PBP

MAY 13 1953

Dr. S. B. Tove
North Carolina State College
Raleigh, North Carolina

Dear Dr. Tove:

I am glad to inform you that we have reviewed your request for renewal of your contract entitled, "A Study of the Effect of the Diet on Lipid Metabolism Using C-14," and we are recommending to the Oak Ridge Operations Office that the contract be renewed for another year. We hope you will be able to make continued progress on this project.

You may expect to hear from the Oak Ridge Operations Office within the near future regarding negotiations of the contract.

Sincerely yours,

Paul B. Pearson

Paul B. Pearson
Chief, Biology Branch
Division of Biology and Medicine

cc: R. W. Cummings, Director of Research

File: A.E.E.

Sherwood ✓
Malone ✓
Smart ✓
Smith ✓
Tove ✓

NORTH CAROLINA STATE COLLEGE
SCHOOL OF AGRICULTURE
RALEIGH

Department of Animal Industry

Return to files
JANUARY 20, 1954 J.W.P.

MEMORANDUM TO: SECTION HEADS, DEPARTMENT OF ANIMAL INDUSTRY
DR. G. H. WISE, ANIMAL NUTRITION SECTION

WE ARE ATTACHING A COPY OF A STATEMENT CONCERNING THE RESPONSIBILITIES, PROCEDURES, AND REGULATIONS GOVERNING USE OF RADIOACTIVITY ON THE CAMPUS. WE SHALL APPRECIATE YOUR CIRCULATING THIS COPY AMONG THE MEMBERS OF YOUR SECTION SO THAT THEY WILL BECOME FAMILIAR WITH THE PROCEDURES AND REGULATIONS AS ESTABLISHED BY THIS MEMORANDUM.

ALL PROPOSED EXPERIMENTAL PROJECTS USING RADIOACTIVE MATERIALS MUST BE APPROVED BY, AND WILL BE UNDER THE SURVEILLANCE OF, THE SAFETY AND HEALTH COMMITTEE APPOINTED BY CHANCELLOR BOSTIAN. THIS COMMITTEE IS COMPOSED OF THE FOLLOWING PERSONS:

DR. C. G. BRENNECKE, CHAIRMAN
DR. C. K. BECK
DR. N. S. HALL
DEAN J. H. LAMPE, EX OFFICIO
DR. A. C. MENIUS
DR. R. L. MURRAY
DR. W. J. PETERSON
DR. F. P. PIKE
PROF. CHARLES SMALLWOOD, JR., SECRETARY

J. W. Pou

J. W. POU, HEAD
DEPARTMENT OF ANIMAL INDUSTRY

JWP:NSR

ENCLOSURE

NORTH CAROLINA STATE COLLEGE

STATEMENT OF RESPONSIBILITIES, PROCEDURES, AND REGULATIONS GOVERNING USE OF RADIOACTIVITY ON THE CAMPUS

Issued by the

COMMITTEE ON SAFETY AND HEALTH

For The

NUCLEAR REACTOR AND RADIOISOTOPES

Raleigh, North Carolina

December, 1953

Section I. INTRODUCTION

For a number of years a Radioisotope Committee on the N. C. State College Campus has borne the responsibility of reviewing and approving the applications of staff members for radioactive isotopes issued by the A. E. C. and of insuring that accepted practices of safety were exercised in the use of these isotopes. The Chancellor has revised the membership of the Committee, enlarged the area of its jurisdiction and increased the scope of its responsibilities.

The Committee is now designated the College Committee on Safety and Health for the Nuclear Reactor and Isotopes. It is the purpose of this statement to describe the responsibilities which have been assigned to the Committee and to outline the procedures by which it will attempt to discharge these responsibilities.

In establishing the new Safety and Health Committee, the Chancellor assigned a central responsibility which is to require in all procedures, uses and disposals of all radioa ctive materials and sources of ionizing radiations on the campus, that sufficient attention be paid to safetv and precautionary measures to insure the protection of all personnel, both of the college and of the public.

In a sense, the Committee cannot discharge this responsibility, for in the final analysis, the ultimate responsibility for safety must rest with the project leasers and the experimental participants.

What the Committee can do, and what it will endeavor to do is to establish basic standards and safety regulations; adopt measures to require adequate experience of personnel bearing project responsibility and adequate equipment and facilities; maintain a system of inventory and inspection designed to assist in elimination of unsafe practices; and, by encouragement, education and constant emphas s on attention to safety, endeavor to instill in campus personnel a conscious, coordinated, and consistent attention to matters of safety in all procedures involving use of radioactivity. Beyond this point, in ways an endless volume of rules would be inadequate to specify, the final responsibility for safety must rest on the project leaders and on each individual who handles radioactivity and radioactive materials.

In addition, the Committee is charged to protect the interests of the College with respect to public reaction and goodwill in matters involving radiation and radioactive materials, and further, to promote, by advice and counsel, the use and application of radioactive materials and sources of radiation on the campus.

This statement is concerned with the procedures by which the central function of the Committee is to be fulfilled. The

corollary functions in part will also be performed if the central objective is successfully achieved, and in part will be realized through attention to individual cases as they arise.

To insure that adequate safety measures are incorporated in the handling, utilization and disposal of all radioactive materials on the campus must be known to the Committee and all proposed uses of such equipment and materials must be known to and approved by the Committee in advance of such usage. To effect this, the Committee will catalogue all radiation sources and radioactive materials now on the campus. The usage thereof will be subjected to inspection. In addition, approval must be obtained from the Committee before any new radioactive materials or radiation equipment are purchased for use on the campus.

Approval of any proposed usage of radioactive material or radiation equipment will be based on the adequacy of safety measures to be exercised and the consideration of public relations. Three principal factors will be considered by the Committee in evaluating the adequacy of the safety risks in a proposed usage: First, the experience and ability of the applicants to cope with the hazards involved in the particular application; second, the adequacy of the facilities and equipment for the proposed usage; and third, the thoroughness and attention given to safety precautions in the proposed experimental manipulations and disposal procedures.

The Committee will establish basic safety regulations, maximum routine radiation tolerance levels, and commonly accepted precautionary measures to serve as the guide to safe operations in handling radioactive materials on the campus, and it will require adherence to these general standards. In addition, the Committee may specify further regulations to be observed for certain types of operations and for particular projects.

To assist operating personnel in observing these safety precautions and to satisfy itself that adequate measures of safety are being practiced, the Committee will employ a Radiological Safety Officer (R.S.O.) to serve in liaison capacity between the Committee and the individual projects on the campus.

Where grossly unsafe practices involving use of radioactivity and radiations, or where practices in violation of established regulations are observed, the R.S.O. has the authority to require cessation of the project until a review can be made by the Committee. If the Committee at any time is not satisfied with the adequacy of safety practices employed in a project, it may require cessation of the project until satisfactory procedures have been adopted.

Section II. GENERAL RESPONSIBILITIES

These responsibilities refer to the radiological aspects involved in the use and disposition of radioactive isotopes, sources emitting ionizing radiation, fissionable materials, fission products, irradiation services and any and all materials or equipment that emit ionizing radiations or particles that are capable of causing ionization of chemical elements. (Hereafter referred to as radiation Materials, Equipment, and Services.) These are defined in Section III with the regulations that pertain to each classification. The Committee will review the regulations to assure that the utility of such radiation materials, equipment, and services are not unduly impaired.

A. Procurement: The Committee has the authority to approve or disapprove the procurement of such Materials, Equipment, and Services. The procedures of and basis for the approval will be reviewed to assure that the responsibilities of the Committee are fulfilled. While it is within the scope of the Committee's activities to give advice and counsel on intended and new uses of these materials, equipment, and services, the criteria for approval must be the radiation and contamination risks and hazards involved and the methods employed. Application forms for the approval to procure radiation substances and services are issued by the Committee. The forms for Materials will carry a serial number of 1; Equipment, 2 through 4; and Services a serial number of 5.

B. Supervision of the Usage and Disposal: The Committee is responsible for seeing that the usage and disposal of radiation materials and equipment are in accordance with such safety standards as are recommended by the Bureau of Standards and the Atomic Energy Commission or its Agencies, or as are established by the Committee itself. The Committee has the right and privilege to require that the user of any radiation materials, equipment, and services as covered in this document, to submit to any and all examinations as it may deem necessary. Such examinations must be concerned with the biological aspects of radiology.

C. Surveillance of the Areas Involved in the Usage and Disposal of Radiation Materials, Equipment, and Services: The Committee establishes procedures for keeping these under as complete surveillance as are needed to minimize the radiological risks and hazards to the safety and health of all involved.

D. A Radiological Safety Officer designated as an Executive Officer, responsible to the Committee, is appointed by the Administration on the recommendation of the Committee. The Radiological Safety Officer is responsible for monitoring the adherence to the regulations of the Committee concerning the radiological safety and health on College property. He is authorized to require adherence to all the regulations of the Committee and in the event of any violation of these regulations, he shall demand cessation of the manipulation or action if con-

tinued after it has been brought to the attention of the person in charge and report such violation to the Chairman of the Committee.

The Radiological Safety Officer will assist and advise campus personnel responsible for radioactivity or radiation projects on matters of radiological safety so as to assure a minimum of delay and inconvenience.

E. Advice and Counsel as to the usage and possibilities of radioactive isotopes, irradiation services and associated subjects is a function of the Committee. Such advice and counsel is separate from and not in connection with the approval of procurement, supervision of use and surveillance of radioactive isotopes, fissionable materials, etc., and the executive duties of the Radiological Safety Officer. It is hoped that the request for advice and counsel will initiate within the several departments and sections of the College. This does not preclude the responsibility of the Committee in initiating such considerations as it deems desirable to promote and accelerate new and extended uses of these materials, equipment, and services within the College, with other colleges and outside agencies.

Section III. REGULATIONS CONCERNING THE PROCUREMENT AND USAGE OF RADIATION MATERIALS, EQUIPMENT, AND SERVICES.

A. Materials: For the purpose of classification, all radioactive substances that are used as part of the reaction or process concerned and essentially change or lose their original form in the reaction or process will be termed materials. Examples of such uses are tracer investigations and isotopic dilution analyses.

1. Application: Application for materials is to be made on a form provided by the Committee. In this form the applicant will clearly set forth the materials desired, the quantity, and the intended usage. While this application does not constitute an affidavit, the Committee will expect adherence to statements referring to the intended use and areas involved. The purchase requisition or a loan request covering the application will carry the Committee authorization number and will be signed by the Chairman of the Committee. The Purchasing Department will be instructed not to issue any purchase orders that do not carry a Committee authorization number and the signature of the Chairman or his delegated representative.

A copy of all applications will be kept in the files of the Committee with a replica in the files of the Radiological Safety Officer.

2. Approval: The Committee as a whole has final judgment as to the approval of an application for materials. Approval by the Committee will be decided by a unanimous vote. In making their decision, the Committee is guided by the report of the Radiological Safety Officer of the College as to the radiological risks and hazards involved and the procedures designed to minimize these risks and hazards.

Granting approval to an application in no way precludes the limitations placed on the College by the Atomic Energy Commission in their Authorization for the Procurement of Radioisotopes from the Commission and its authorized suppliers.

This General Authorization does not apply to (a) human experimentations and (b) experimentations involving widespread distribution of Radioisotopes. In these cases, additional approval must be sought from the Isotopes Division of the Atomic Energy Commission.

3. Responsibility of the Committee: Any alteration of an approved request beyond its approved usage that may involve additional radiological risks and hazards must be reviewed by the Committee at large and treated in the same manner as a new project. A renewal of an application that involves no change that alters the radiological risks and hazards present in the original application may be approved by the Chairman or his delegate. The judgment as to whether additional radiological risks or hazards are involved rests with the Committee.

The Committee will require adherence to the safe practices set forth in Section IV at all times. Evidence of failure to comply with these safe practices shall cause the Committee to demand cessation of all operations and not allow the project to be renewed until they are assured of compliance with the established safe practices. It will decide with the R. S. O. what inspection, monitoring, spot checking, and other investigation of the use of radioisotopes on the campus should be made.

4. Responsibility of the Radiological Safety Officer (R. S. O.): He is responsible for making investigations as to the use of radioisotopes and reports at periodic intervals to the Committee the results of such investigations.

He may investigate the use and method of handling of any isotope on the campus at any time. He will point out to the investigator any unsafe conditions or practices that he may observe. In the event that the practices are not appropriately modified, he may demand cessation of the project pending subsequent action of the Committee. If cessation of a project is demanded, the R.S.O. must report the incident to the Chairman at once.

He must keep adequate records on observations, inspections, and investigations of the use of radioisotopes on the campus. He will investigate any reported accident or spill of radioisotopes on the campus.

5. Responsibility of the Applicant: He must furnish information requested by the Committee and by the R.S.O., both at the time of original approval and at any subsequent time requested, regarding the proposed usage of isotopes and the procedures expected to be used. This information, at the time of original approval, may include a complete "cold-run" demonstration of the anticipated procedures.

He must present for Radioisotope Committee approval any change in the use of a radioisotope beyond that approved by the Committee.

He must report as promptly as possible to the R.S.O. any accident involving radioactive isotopes or any spill of radioisotope on the campus.

He is responsible for the adherence to the safe practices set forth in Section IV.

B. Equipment: Class I: For the purpose of classification, Equipment Class I is defined as materials or substances which emit ionizing radiations or particles which may cause the ionization of chemical elements and whose use is dependent upon this emission. The equipment may be an integral part of a process or reaction but, in itself, does not enter into the reaction and thus does not change in form or composition, other

than is associated with the emission of the radiation or particle. This classification is based on intended usage and has no bearing on the expected life of the materials or substances. Fissionable materials are excluded from this classification and are entered as Equipment: Class II.

Examples of such equipment are: sources of radiation for standardizing instruments, gamma sources such as Co 60, alpha sources Po 210, and neutron sources as Ra-Be or Po-Be.

1. Application: Application is to be made in writing on the form designated for this purpose by the Committee. The Applicant in this case will be the department or administrative section. The application will set forth data pertaining to the kind of materials and substances, the amount (radiologically), the intended usage and additional information as is required. This application is to be transmitted to the Chairman of the Committee for presentation to the Committee as a whole. The original copy will constitute part of the Committee's file and a replica shall reside in the file of the R.S.O.

The purchase requisition or a transfer request pertaining to the equipment must carry the approval number of the Committee and the signature of the Chairman or his delegate before the order will be executed.

2. Approval: The Committee as a whole has final judgment as to the approval of an application for Equipment; Class I. In making their decision, the Committee is guided by the report of the R.S.O. as to the radiological risks and hazards involved in the use of such materials and substances.

Granting approval to an application in no way precludes the limitations imposed upon individuals by the A.E.C. or its Agencies in purchasing any materials and substances that would be included as Equipment; Class I.

3. Responsibility of the Committee: Prior to approval, the Committee will classify such Equipment; Class I into two (2) categories: (a) that equipment needing a relatively small amount of surveillance and (b) that equipment needing relatively close surveillance.

If approval is granted, the Committee will stipulate the type and degree of surveillance that such equipment requires.

It insists that when equipment is in the possession of the College, an individual be assigned as the responsible person. The Committee must approve the individual to whom this responsibility is delegated. If any change in responsibility occurs, the Committee will keep a record of the change and reasons thereto. It may require that the person responsible for equipment keep a log of its usage and that this log be opened for inspection by the Committee or its delegate.

The Committee requires that the use of such equipment conforms to the safe practices set forth in Section IV. In the event of any violation of these practices, the Committee shall demand cessation of the operations.

4. Responsibility of R.S.O.: He is to inspect and monitor according to the decision of the Committee each item of Equipment; Class I and report to the Committee at the next meeting after the inspection.

He may investigate the use and location of such equipment at any time and request information on its use from the individual in charge.

He may demand cessation of any manipulation if, in his judgment, unsafe practices are continued after being brought to the attention of the person in charge. He must report at once to the Chairman of the Committee any unsafe conditions and practices known to persist. Resumption of manipulation by the user may take place only by the consent of the Committee or its agent.

5. Responsibility of the person in charge of the equipment: He must furnish information requested by the Committee and the R.S.O. at any time as to the location and use of the equipment.

He is to report to the R.S.O. any change in the location or responsibility associated with the equipment.

If required by the Committee, keep a log of the use of the equipment and permit the Committee or its delegate to inspect the log at any time.

He must request approval for any new or extended use that increases the radiological risks or hazards involved.

He must report to the R.S.O. any change in the form or structure of the equipment that would increase the radiological risks or hazards involved in its use or location.

He is responsible to see that the usage complies with the regulations on safe handling set forth in Section IV.

C. Equipment: Class II: For the purposes of Classification, Equipment. Class II is defined as any aggregate or collection of fissionable material into an assembly such that a nuclear chain reaction can be initiated; a nuclear reactor of any type.

1. Application and Approval: The Nuclear Reactor, now in operation, has been approved by College Authorities; hence no further sanction is needed by the Committee. Future applications for the approval of the Committee to initiate negotiations with the proper authorities for the construction and operation of Equipment; Class II shall be made in person to the Committee at large.

2. Responsibility of the Committee: It is recognized that a Reactor is a unique instrument in that it is a source of radiation as well as products of radiation and products of the nuclear fission. This requires that particular attention be directed to the relationship of the Committee and its R.S.O. so that sufficient freedom of action may be had to assure its rightful role in the College.

The Committee will require adherence to established safe radiological practices and procedures in any manipulations of a Reactor. It will require adherence to the regulations and orders pertinent to radiological safety as set forth in any contract between the College and the A.E.C. that may be pertinent.

It takes the position that nothing in a contract between the College and the A.E.C. concerning a Reactor precludes the responsibility of the Committee on Safety and Health for the operation of the Reactor in accordance to the radiological safe practice standards that it may establish. In the event of violation of these practices, the Committee may demand cessation of the operations that are involved in the violation.

The Committee states that the laboratories and the Reactor room in the Reactor building are subject to the same requirements and conditions of radiological safe practices, monitoring, and inspection as are other laboratories on campus.

It requires that a monthly report be made by the Director of the radiological exposure of all persons receiving an exposure greater than daily tolerance in any 24-hour period or weekly tolerance in any 7-day period. This report shall include the record of all visitors receiving these exposures. It requires the immediate report to the Chairman of any person or persons receiving a weekly maximum permissible radiological dosage in any 7-day period. It directs the R.S.O. to conduct an investigation of the causes of the exposure and submit a formal report to the Committee on his findings and the measures taken (if any) to prevent recurrence of such an exposure. This report will be submitted also to the Department Head involved and a copy shall be attached to the permanent personnel monitoring record of the individual.

It requires that a Safety Officer be informed of all operations and manipulations of the Reactor. He will designate the exposure time for all areas that are in excess of 6.25 mrem per hour.

3. Responsibility of the R.S.O.: He has access to an accurate log of the exposure of all individuals. He will investigate all radiation exposures exceeding daily tolerance in any 24-hour period and submit reports of this

investigation to the Department Head involved and to the personnel monitoring record of the individual.

He is to advise the Committee as to the type of personnel monitoring that should be employed.

He may demand the cessation of of operations and manipulations if, in his judgment, unsafe practices are continued after being brought to the attention of the person in charge. He must report at once to the Chairman any unsafe conditions and practices known to persist. Resumption of these practices and manipulations may take place only by the consent of the Committee or its agent.

He has access to the log of the radiological content of all afflux from the Reactor Building. It is his responsibility to notify the Committee in case the level of radioactivity in the gaseous or liquid afflux is greater than tolerance values as set forth in Section IV.

He may investigate the operation and manipulation of the Reactor at any time.

4. Responsibility of the Director of the Reactor: He is to be a member of the Committee on Safety and Health.

He will supply the Committee with all information requested concerning the radiological safety and health provisions of the Reactor. He is to keep radiological exposure records of all persons concerned with the operations and manipulations of the Reactor and will show these records to the Committee upon request. He is to report immediately to the College R.S.O. any exposure in which a person or persons have received a daily maximum permissible exposure in any 24-hour period or a weekly exposure in any 7-day period.

He will inform the College R.S.O. of all operations and manipulations. He is to report to the College R.S.O. any accidents involving exposures or spills that result in radiological risk and hazard.

He determines exposure levels for various operations and manipulations and determines the exposure time for the personnel involved. He will not, except in case of emergency, permit greater exposure to personnel than is consistent with the standards of radiological safe practices set forth in Section IV. He is to require that all personnel adhere to the safe practices as set forth in Section IV.

He will not irradiate or cause to be irradiated any materials, substances, animals, or plants unless approval of the Committee based on radiological risks and hazards has been obtained. He is to keep, or cause to be kept, a record of all such irradiations and permit the Committee or its agent to review this record.

He will submit to the Committee for approval the plans of any experimental project or projects involving the opening of the Reactor shield. This does not include routine operations and manipulations of the Reactor nor each opening of the shield in the course of performing the experimental project that has received Committee approval. He is to notify the Committee in writing of any change in the experimental procedures or material of an approved project that increases the radiological risks or hazards.

D. Equipment; Class III: For the purpose of Classification, Equipment; Class III shall be defined as all equipment in which a difference in potential is used to produce ionizing radiations. An example of such equipment is X-ray equipment.

1. Application and Approval: At this time the Committee does not desire to exercise any jurisdiction over the procurement of such equipment, but this does not preclude a later decision to exercise such jurisdiction.

2. Responsibility of the Committee: It is the responsibility of the Committee to inventory all such equipment in the possession of the College or on College property and used by College personnel. This equipment will be further classified as to its degree of potential hazard to the radiological safety and health of personnel into two categories (a) little or no risk or hazard and (b) appreciable risk or hazard.

It stipulates the degree of surveillance which the R.S.O. shall maintain in connection with each of the items on the inventory.

It will have designated, subject to its approval, a person who is responsible for the operation and manipulation of each item of equipment and considers this person to be responsible for all operations and manipulations unless notified to the contrary in writing. It may require that a log of the operations and manipulations be kept and that this log be subject to the inspection of the Committee or its agent. The Committee requires that the person in charge use this equipment so as to conform to the safe practices set forth in Section IV. In the event of any violation of these practices, the Committee shall demand cessation of the operations.

3. Responsibility of the R.S.O.: He inspects and surveys each item in accordance with the stipulations of the Committee.

He may demand cessation of any manipulation if, in his judgment, unsafe practices are continued after being brought to the attention of the person in charge. He reports at once to the Chairman of the Committee any unsafe conditions and practices known to persist. Resumption of manipulation by the user may take place only by the consent of the Committee or its agent.

He keeps records of all surveys and inspections that are made.

4. Responsibility of the Person in Charge: He is to notify the Committee of any change in the equipment that increases the radiological risks or hazards involved.

He is to keep such records as may be required by the Committee and make these records available to the Committee or its agent.

He will notify the Committee in writing of any change in his responsibility to the equipment.

He will permit the R.S.O. to inspect and survey the equipment at any time.

He is responsible to the Committee for assuring that the equipment is utilized in accordance with the established standards of safe practices.

E. Irradiation Service: Radiation Service, in this instance, shall be limited to the use of the Reactor on the College campus as a source of ionizing radiation.

1. Application: The applicant will set forth in writing details of the requested service as required on the form designated by the Committee for this purpose. The original application will be directed to the Committee with copies for the College R.S.O. and the Director of the Reactor.

Procurement arrangements will be made directly with the Director of the Reactor. If a purchase requisition is issued, it will carry the approval number of the Committee and the signature of the Chairman.

2. Approval: The responsibility of approval rests jointly with the Committee and the Director of the Reactor and/or his delegates. In case either rejects approval, the application shall be considered as not approved as set forth in the application.

3. Responsibility of the Committee: It requires adherence to established standards of safe practices as set forth in Section IV that pertain to such operations and manipulations.

It requires an immediate written report of any over exposure of persons or persons involved in the operations and manipulations.

It may require the presence of a Safety Officer at the time of initiation and completion of the service and may demand that he determines the exposure of areas and designates the working time of each person in specific areas.

4. Responsibility of the R.S.O.: He is to acquaint himself with the exact operations and manipulations pertaining to the service.

He may demand cessation of any manipulation if, in his judgment, unsafe practices are continued after being brought to the attention of the person in charge. He must report at once to the Chairman of the Committee any unsafe conditions and practices known to persist. Resumption of manipulation by the user may take place only by the consent of the Committee or its agent.

5. Responsibility of Applicant: He is to supply the Committee and the Director of the Reactor all information requested relative to the desired service.

He will comply to the procedures of safe practices as set forth in Section IV and with any and all additional restrictions imposed by the Committee relative to the radiological risks and hazards involved in the irradiation service.

Section IV. GENERAL RADIOLOGICAL SAFETY PROCEDURES

The maximum permissible level for total body radiation exposure shall be 0.3 rem per week. This shall not be acquired at a rate greater than 0.05 rem per day except where operating conditions require a higher rate for a special project. When such a higher rate is to be used, a Safety Officer must be present during the operation.

The maximum permissible level for exposure to a limited portion of the body shall be the same as for whole-body exposure except that the hands and feet may be exposed to a maximum of 1 rem per week where necessary for operational reasons.

No individual shall knowingly expose himself or cause others to be exposed to levels of radiation greater than those delineated above except in cases of extreme emergency or medical x-ray examinations or treatment. The lowest practical daily exposure should be striven for in every operation. The exposure limits listed above represent the total additive exposure from all components of radiation involved.

The relationship between r, rep, and rem is considered to be as given in the following table. Maximum values of neutron flux for an eight hour day exposure are also given.

Type of Radiation	Relative Biological Effectiveness	rem/week	rep/week	Maximum Neutron Flux for an 8 hr. day exposure
X or Gamma	1	0.3	0.3	
Beta	1	0.3	0.3	
Protons	10	0.3	0.03	
Alpha	20	0.3	0.015	
Fast Neutrons	25	0.3	0.012	20 $n/cm^2/sec$
Thermal Neutrons	5	0.3	0.06	500 $n/cm^2/sec$

RADIATION AREAS:

The term Radiation Area shall be used to designate any area in which precautions against radiation exposure are required. Radiation areas shall be plainly marked and where personnel monitoring equipment is required, the marking shall designate this requirement.

CLEAN AREAS:

It is recognized that within some buildings that are extensively using radiation materials or equipment there will exist a need for protecting certain areas from radioactive contamination. Examples of such areas are low level counting rooms, administrative offices, photographic dark rooms, etc.

It is proposed that these areas be designated "Clean Areas," and that provisions be made for monitoring all equipment coming into a Clean Area after having been exposed to radiation or radioactive materials in another part of the building or campus. It must be emphasized that the tolerance levels on the monitoring of suspected equipment to be brought into a Clean Area are more strict than those imposed for personnel protection due to the possibility of cross contamination in these low level areas. It should be noted that the monitoring of such equipment must be carried out in a place where the background is sufficiently low to reveal minimal levels of activity.

The limits of contamination for articles to be admitted to a Clean Area on the basis of a 100 cm^2 area are as follows:

Loose contamination detectable by smears:

	d/m
Alpha	0
Beta Gamma	0

Maximum total contamination:

	d/m
Alpha	100
Beta Gamma	200

Brooms, mops, floor polishers, and other janitorial equipment used in Clean Areas shall be set aside for use in those areas only.

PROTECTIVE CLOTHING:

Suitable gloves are to be recommended whenever hand contamination is probable. Rubber gloves shall be worn when handling open vessels containing alpha material or when handling any equipment of comparable hazard. Rubber gloves are to be preferred for cases where liquid contamination may be present or where radioactive dust might filter through a cloth glove. Rubber gloves are to be cleaned, if practical, before removal. All gloves are to be stored and handled so as to prevent contamination of the inside surfaces.

Whenever known "hot" jobs are to be conducted, i.e. where the probability of radioactive contamination is high, coveralls or lab coats, gloves, and shoe covers should be worn to protect personal clothing while involved in the job and to confine contamination to the "hot" area by removal of such clothing prior to leaving the area.

EATING AND SMOKING:

Eating, storing, or preparation of food in a laboratory or room where active materials are handled will not be permitted.

It is strongly recommended that smoking in radioactive laboratories be prohibited. This is in recognition of the inhalation hazard and is consistent with good chemical practice.

CONTAMINATION CONTROL:

Personnel:

All persons while working with radioactive materials wherein hand and shoe contamination is possible, are to

Wash hands thoroughly before eating, smoking, or leaving work.

Wash rubber gloves before removing from hands unless the radiation level requires immediate removal.

Utilize the available equipment to assure that decontamination has been effected.

No work with long lived alpha and beta-gamma emitters, in any chemical or physical form, is to be performed by a person having a break in his skin below the wrist unless gloves known to be clean on the inside are worn.

The pipetting by mouth of liquids containing appreciable radioactivity is forbidden. The term "appreciable" in this connection is taken to include all radioactivity other than naturally occurring trace amounts present in water, potassium compounds, etc.

Area:

All areas in which there is radiation in excess of 6.25 mrem/hr shall be physically isolated and appropriate signs posted to prevent persons from entering the area without being aware of the radiological hazard. Signs having the Health Physics symbol will be standard for radiation hazards. The symbol will also be used to distinguish radioactive source containers, contamination areas, hot sinks, barriers, etc.

Loose contamination should not be tolerated on exposed surfaces and should be removed as soon as possible. Small amounts of fixed contamination may be unavoidable at times. Maximum limits of 1000 d/m per 100 cm^2 of alpha, and 1 mrep/hr (at 2 cm) of beta-gamma shall be established. After determining that the fixed contamination falls below these maximum values, the area should be given one or two coats of a good, hard surface coating. The same standards of contamination control shall apply to tools and equipment. Signs and control for contaminated surfaces, areas or equipment shall be instituted to prevent occurrence of a health hazard or the spread of contamination.

All spills of radioactive material must be cleaned up promptly. Cleaning responsibility shall rest on the individual(s) working in the area involved, and a survey shall be made after cleaning to verify that the cleaning has removed the radioactive material(s). A Safety Officer shall be notified of all spills or incidents involving possible contamination.

STORAGE, TRANSPORTING, AND HANDLING OF RADIOACTIVE MATERIALS:

Quantities of long lived alpha emitters or similar hazardous substances having an activity greater than 1 microcurie shall be securely covered during storage and kept in an adequately protected and ventilated location.

All transfer of materials between hoods and storage devices must be done in such a manner as to avoid the possibility of spillage or breakage. Double containers are to be recommended in such manipulations.

Any work with materials susceptible to atmospheric distribution (i.e. dusting, spillage, vaporizing, effervescense of solution, etc.) of long lived alpha products or any emitter of similar hazard shall be performed in adequate hoods. Suitable instrumentation for each type of material to be used must be available and in operating condition in the area in use. "Suitable" in this case will mean that the instrument will easily detect maximum permissible levels of contamination, radiation level, air hazard, etc., as are associated with the material(s) involved.

It is recognized that in the course of some experiments there will be a need for the handling of "hot" equipment or the reworking of "hot" equipment to conform to the requirements of the experiment. Such equipment should not be taken into non-radioactive areas until (1) all personnel within such areas are notified of the intent to bring in the material, (2) complete protective measures have been incorporated to assure minimum contamination to the area and to assure that any atmospheric distribution of the material resulting from the handling or reworking will be below the maximum permissible concentration value for the airborne material. It is apparent that certain equipment will conform to the safe limits associated with fixed contamination (i.e. radiation hazard), but the same material dispersed in the atmosphere would be above the safe concentration values for the material ingestion.

All areas where radiation materials and equipment other than waste are to be stored must be clearly marked with the Health Physics symbol and information pertinent to the isotope(s) indicated. (Radioactive element(s), quantity, half life,

radiation level at surface and date, and its location.) Such markings shall be placed in such a position that there will be no radiological risk or hazard to any personnel while reading it.

WASTE DISPOSAL:

General:

Most of the radioactive wastes that will be produced at State College must be stored on campus for radioactive decay or transported elsewhere for permanent disposal. The college will establish a central area for storage of radioactive materials.

It is necessary to keep the volume of such waste as small as practicable. In order to make full use of concentrating techniques and of the natural decay characteristic of radioactive substances, it is important that waste be segregated and labeled as far as practical according to type.

It shall be the responsibility of all individuals producing radioactive wastes to be aware of disposal limits and to conform to all requirements pertinent to safe disposal. In particular, efforts should be made to limit the volume of radioactive wastes and to furnish information concerning the nature of wastes being collected for subsequent storage, i.e. (1) Isotope(s), (2) Half life, (3) Estimated Activity in waste (M.C.), (4) Date of estimation, (5) Radiation level at the surface of the container, and (6) The instrument used in determining the surface radiation level.

It is strongly recommended that all radioactive waste materials or equip ent be removed from the working area as rapidly as possible. During the time that these materials or equipment remain in the working area, shielding should be utilized to minimize the radiological risks and hazards.

Gaseous Wastes and Air Contaminants:

If the radioactive waste(s) to be disposed of is (are) in the gaseous state, the concentrations of affluents at all locations to which people have access without time limitation shall not exceed the permissible 24 hour ingestion or inhalation limits for the isotope(s). Wee ly average concentrations shall not exceed 10% of these limits within the working area or building or 1% of these limits at all points outside the working area.

The discharge of particulate radioactive contamination is to be avoided by the use of filters or suitable experi-

mental techniques. All operations with radioactive materials and equipment shall be carried out in such a manner as to minimize contamination of the air.

It is recognized that Xe^{133} will be one of the gaseous fission products developed with the nuclear reactor. The maximum permissible concentration that will be allowed to be released at the top of the stack shall be 2.0×10^{-6} µc per cm^3 of air.

Solid Wastes:

If the radioactive waste(s) to be disposed of is(are) in the form of a solid, it shall be placed in properly labeled containers to be stored in a "hot" waste storage area until natural radioactive decay has reduced the contamination to background level or until the waste may be sent to a "hot" waste disposal area such as O.R.N.L.

It is desirable that short lived materials be kept separate from those of long half lives where feasible. Whenever possible the nature of the material being disposed of should be fully described (as indicated above) on a tag or sticker attached to the container.

Waste cans for solid radioactive waste shall be removed when the maximum safe radiation level or volume is reached. The safe radiation level is defined as the level such that no one can receive an exposure of more than 50 mrem per 8 hour day at the surface of the container.

All waste packages shall be carefully wrapped to preclude the possibility of external contamination. Glassware and other noncombustible material should be packaged separately.

Liquid Wastes:

Liquid waste materials are of such a nature that the responsibility for conforming to safe disposal practices rests on the individual user of radioactive materials. The feasibility of a safe liquid waste disposal program will be based upon the methods used by the individual user of radioactive isotopes to get rid of his waste. In some instances, the holding of liquid waste for radioactive decay will be preferable to releasing the material into the sewage. Whenever the material is to be released into the sewage the individual should ascertain that the radioactive concentration will comply with the limitations established by the Safety and Health Committee.

Known dilution factors within the sewage system will be useful to the individual user, but it is apparent that the daytime dilution factor within the sewage network will not

be the same as night time dilution factors. The appropriate dilution factor for the time of release should be utilized where practical. The possibility of several individuals using the sewage system dilution factor simultaneously exists. To assure that this problem does not arise, the individual user shall notify the R.S.O. whenever he will include the campus sewage system's dilution factor in his liquid waste disposal to conform to maximum concentration values. If a conflict on time of disposal exists, the R.S.O. will arrange another time for the project involved.

In addition to using the sewage system's dilution factor, a dilution factor of at least 100 should always be used at the point of release. This will minimize the possibility of a slug effect where a material might remain concentrated all the way through the sewage system.

Wastes that are liquid in form or that are to be reduced to a liquid for disposal shall be governed by the following procedures:

The maximum concentration of radioactivity released from the N. C. State College sewage system shall not exceed 3×10^{-5} curies/cc of water. The average concentration over a three month period shall not exceed 3×10^{-6} curies/cc of water. The total activity released shall not exceed 1.5 curies per year.

It is recognized that a monitor of liquid waste effluent will not be able to detect certain low energy beta emitters such as C^{14}, S^{35}, etc. Rigid control of wastes from such isotope usage will be maintained. The R.S.O. shall first be notified of any significant waste material to be released, estimate of total radioactivity, concentration of the solution, and time of release into the sewage system.

Whenever it is seen that the release of any liquid waste will exceed the maximum radioactive concentration limits on N. C. State College sewage, the waste shall be either (1) diluted below the maximum concentration limits and then released, (2) held for radioactive decay to a point below the maximum concentration limits and then released, or (3) prepared for shipment to a "hot" waste disposal area such as O.R.N.L.

All liquid waste having a total radioactivity content greater than 1 millicurie shall be reported to the R.S.O.

PERSONNEL MONITORING:

A film badge as prescribed by the Safety and Health Committee shall be worn by all persons entering a radiation area where a daily whole-body exposure of 10 mrem or more is possible. Signs will be posted designating the areas in which persons are required to wear personnel monitoring equipment. The Safety and Health Committee will require designation of such areas.

The wearing of two pocket ionization chambers in addition to the film badge may be required in cases where a day by day indication of exposure is desirable.

Pocket chambers and film badges are not to be tampered with in any way and are intended primarily for monitoring the person to whom they are issued. If personnel monitoring equipment is needed for special experimental purposes, arrangements should be made with the R.S.O.

Personnel Monitoring Records will be maintained.

Visitors:

The term "Visitors" is used to designate all persons for whom personnel monitoring equipment is not provided on a routine basis, including employees of State College, as well as visitors from the outside. Visitors shall be given the same personnel monitoring coverage as regular personnel working in a radiation area.

Responsibility for the Enforcement of Personnel Monitoring Procedures:

It shall be the responsibility of all individuals to wear personnel monitoring equipment within the radiation areas as defined above. Supervisors are to assure compliance in the matter for the people for whom they are responsible.

TRAINING IN HEALTH PHYSICS

In order to guarantee effective compliance with the procedures and requirements outlined above, the R.S.O. shall provide general indoctrination in regard to radiation hazards, contamination hazards, and protective methods to all personnel working with radioactive materials or who will be working in a radiation area. It shall be the responsibility of department heads to ascertain that personnel working in these areas be indoctrinated in these procedures and if not, have such personnel avail themselves to the R.S.O. for such indoctrination prior to working in such areas.

UNITED STATES
ATOMIC ENERGY COMMISSION

In Reply
Refer to: OR:JER

Oak Ridge, Tennessee
March 1, 1954

Dr. S. B. Tove
North Carolina State College
School of Agriculture
Raleigh, North Carolina

Subject: RENEWAL OF CONTRACT NO. AT-(40-1)-1324

Dear Dr. Tove:

We are enclosing a "Revised Guide For the Submission of Research Proposals", outlining the policy of the Atomic Energy Commission on the support of research at colleges and universities. Under this policy, the institution will submit a total cost budget and indicate what proportion of the total cost the Commission is requested to support. The actual going rate of overhead or indirect cost should be included, with a brief explanation of the method of computing these costs. Submission of the total costs of the project will assist the Commission in determining its level of support, based on the degree of interest the project has to the activities of the Commission.

Any contemplated renewal of your contract should be submitted in conformance with the policy outlined in the enclosed guide. The renewal proposal and the progress report must be submitted in sextuplicate, each as a separate document, to the Operations Office.

It is necessary that the renewal proposal be endorsed by a responsible administrative officer of the institution. Also, a statement of the expenditures, indicating the expenditures up to the date of the submission of the proposal and an estimate of the expenditures to be incurred during the remaining portion of the current contract period, should be enclosed.

The proposal for the renewal of your contract should reach us not later than April 1, 1954. It should be addressed to:

U. S. Atomic Energy Commission
Research and Medicine Division
Post Office Box E
Oak Ridge, Tennessee

Your cooperation in submitting renewal proposals on time and complete, will assist us in securing an early review and decision.

Very truly yours,

Kenneth Kasschau
Director
Research and Medicine Division

Enclosure:
Revised Guide for the Submission
of Research Proposals

Rounsaville:mah

CC: J. G. Vann
R. W. Cummings

U. S. ATOMIC ENERGY COMMISSION

DIVISION OF RESEARCH

DIVISION OF BIOLOGY AND MEDICINE

DIVISION OF REACTOR DEVELOPMENT

REVISED GUIDE FOR THE SUBMISSION OF RESEARCH PROPOSALS

February 8, 1954

INTRODUCTION

Through its Divisions of Research, Biology and Medicine, and Reactor Development the Atomic Energy Commission, under authority of the Atomic Energy Act of 1946, contracts with independent institutions for research in fields related to atomic energy. Under these contracts the universities, colleges, industrial laboratories, and other research institutions contribute to scientific progress in fields related to the development and use of atomic energy. Applied research directed towards specific goals of practical accomplishment and basic research which discovers new principles and broadens our understanding of physical and biological phenomena are both essential. The first serves the immediate requirements of the program, and the second underlies future capabilities. Both types of research contribute to the training of scientific manpower.

POLICY

In selecting projects for support under these programs the Atomic Energy Commission recognizes the importance of independent research, and its policy is to assist competent investigators working in an environment favorable to productive research along lines which the scientist himself considers promising and consistent with the interest of his institution. Usually his institution continues to make its normal contributions to his research expenses. At the same time it must be emphasized that the AEC only participates in programs of research which are related to its statutory responsibilities.*

*These responsibilities are defined in part by Section 3(a) of the Atomic Energy Act of 1946 as follows:

"(a) Research Assistance. - The Commission is directed to exercise its powers in such manner as to insure the continued conduct of research and development activities in the fields specified below by private or public institutions or persons and to assist in the acquisition of an ever-expanding fund of theoretical and practical knowledge in such fields. To this end the Commission is authorized and directed to make arrangements (including contracts, agreements, and loans) for the conduct of research and development activities relating to --

(Footnote continued on page 2)

THE COST-SHARING LUMP-SUM CONTRACT

A type of arrangement which the Commission has adopted for assisting research in independent institutions is the lump-sum cost-sharing contract. These contracts can be used when the annual cost to the AEC is less than $100,000 and can be estimated with reasonable accuracy in advance. In consideration for the institution's carrying out the agreed investigations and submitting a satisfactory report the AEC agrees to pay a lump sum based upon an agreed part of the estimated total cost. The prospective contracting institution bases its proposal upon a clearly delineated level of activity which is used as a basis for estimating the cost. In making its proposal the institution states what part of the total costs it is prepared to bear and what part it requests from the AEC.

Title to equipment acquired for carrying out the project will normally vest in the contractor unless provision is made to the contrary.

COST CONTRACTS

Projects requiring higher contributions from the AEC or those of which the costs cannot be estimated with reasonable accuracy are financed under cost-type contracts. Information about this type of arrangement can be obtained by consulting the AEC.

CLASSIFICATION

Experience has indicated that the great majority of projects fall in unclassified areas, and can therefore be conducted without security restrictions. If contact with classified areas appears to be advantageous clearance may be requested. Where there is chance that classified information may be developed by the work, clearance is required for the senior investigator. In some few cases it may be necessary for all persons associated with the project to have security clearance. When it appears to the senior investigator that information is being developed that may be subject to classification he should immediately notify the AEC. The AEC will determine what classification, if any, will apply to the information. The continuation of a project found to involve classified information will be subject to mutual agreement of the AEC and the contractor.

(Footnote continued from page 1)

(1) nuclear processes;

(2) the theory and production of atomic energy, including processes, materials, and devices related to such production;

(3) utilization of fissionable and radioactive materials for medical, biological, health, or military purposes;

(4) utilization of fissionable and radioactive materials and processes entailed in the production of such materials for all other purposes, including industrial uses; and

(5) the protection of health during research and production activities."

PROPOSALS

Proposals for research contracts are usually initiated by the scientist interested in doing the work. After reaching an understanding with responsible officers of his institution he may desire to discuss the project informally by correspondence or by personal visit with a member of the appropriate division of the Washington staff before submitting a formal proposal.

Formal proposals should cover the following points insofar as they are applicable:

1. Title of the project.

2. The institution and department in which the work will be done.

3. Scientific background including literature relevant to the proposal, the significance, and the motivation. If the proposal is for continuation of work already in progress the extent of present support should be stated identifying amounts received from federal agencies.

4. Scientific scope of the proposed research, its objectives, its relation to present knowledge and to comparable work in progress elsewhere, and a plan of accomplishments for the first year's work.

5. Scientific Personnel. Give the name, highest academic degree, position in the institution, scientific experience, publications and accomplishments of the senior investigator (the individual who will actively direct the research program) and of each regular staff scientist who it is proposed will engage in the work. Indicate the approximate fraction of the time of each to be devoted to the project during each period of the year. Scientific personnel to be newly employed for the project should be so designated, and professional records given if possible.

6. Other personnel. The number of persons of each sub-professional grade and the fraction of the time of each to be devoted to the project should be listed. Graduate student employees should be identified as such if their thesis is to be related to the project.

7. Other Financial Assistance. If assistance for this or other activities involving the same personnel or facilities is to be proposed to, or received from other federal or non-university sources the extent of that assistance should be clearly stated, and the interplay of the arrangements should be fully explained.

8. Materials, Equipment and Facilities. List those already available for the work and justify the need for major items to be procured.

9. Travel and other items. Explain the purpose of the proposed travel, and of any other major items in the budget. Travel rates and the use of contract funds for attendance at regular scientific meetings should conform with the policy of the institution in the use of its own funds for these purposes.

55
F
490

10. Budget. This should list in detail all items of cost necessary to carry the project for one year or for the duration of the project if less than a year. It should include: a list of the individual salaries attributable to the project, supplies and services, equipment (defined as things individually costing more than $500 which will retain their utility for more than a year), travel, communication and publication, and the indirect costs allocable to the project. The basis for computing the indirect costs should be briefly explained. The budget should not include the stipend of fellows. All salaries chargeable to the project should be in accord with the established policies of the institution, or, if not, an explanation should be submitted.

11. Amount requested. A statement of the part of the total amount listed in the budget which the institution is prepared to bear, and the amount requested from the AEC, and a statement of any other sponsors of the project with the amounts contributed by each.

The proposal should be signed by the Senior Investigator, endorsed by a responsible administrative officer of the institution, and sent in sextuplicate to the Division of Research, the Division of Reactor Development, or to the Division of Biology and Medicine, U.S. Atomic Energy Commission, Washington 25, D.C.

NOTIFICATION

Since proposals are usually reviewed by a scientific panel or advisory committee before being acted upon, a period of four to eight weeks may elapse before a decision is reached. The Senior Investigator will be notified when his proposal is acted upon by AEC Washington. If approved for AEC assistance it will be forwarded for contract negotiation and administration to one of the AEC Offices of Operations. The AEC assumes no financial obligation until the contract has been executed.

PAYMENTS

The schedule of AEC payments will be arranged at the time of contract negotiation.

REPORTS

Satisfactory completion of a contract will include submitting a satisfactory report of the investigation. The schedule of reports will be arranged at the time of contract negotiation. Usually an annual progress report is sufficient. When a contractor desires his contract to be renewed the progress report will cover the period up to the date of the renewal request and will be submitted in sextuplicate as a separate document with that request. The progress report should briefly describe the scope of the investigations undertaken and the significant results obtained. It should also explain any significant differences between the actual level of activity (expressed in the various categories of man months, facilities procured, travel performed, et cetera) and that contemplated in the contract. The technical reports and articles prepared for publication during the period covered should be listed with bibliographic

references. Reprints or preprints of all such material should be appended and material contained in them need not be duplicated in the report. Publication in the open literature is recognized as the normal and most desirable means for reporting the findings of unclassified research and it is not intended, except in unusual circumstances, to require contractors to report results in detail before they are ready for publication.

200-WORD SUMMARY

Immediately after a contract is negotiated a summary of 200 words, more or less, covering the purpose and scope of the project should be sent in triplicate to the AEC, Office of Operations. After each renewal of the contract the 200-word summary should be revised to include the significant results and conclusions of the former year's work and a statement of the scope and objectives for the following year. Since the summaries may be given wide circulation they should be prepared with careful thought to both content and style.

RENEWAL PROPOSALS

Contracts usually cover a period of one year but they may be extended year by year. To insure continuity, renewal requests in sextuplicate should be submitted to the AEC Office of Operations not later than three months nor earlier than six months before the expiration of the contract. Early submission may result in early approval. The renewal proposal should include the same type of information as that required under items 1 to 11 above unless the information is already contained in earlier proposals or in the accompanying progress report. Any contemplated change in program or scope for the renewal period should be clearly explained and the cost estimated should be based upon past experience. Any difference in the scope of the work during the current contract period from that contemplated in the contract, as brought out in the Report, may be reflected in the amount requested for the ensuing year. If no new funds are required the contract may be renewed without funds. A proposal for such renewal should state the scope of the work proposed for use of residual funds.

February 8, 1954

GPO 869739

File: AEC

UNITED STATES
ATOMIC ENERGY COMMISSION

In Reply
Refer to: OR:JER

Oak Ridge, Tennessee
May 11, 1955

Dr. S. B. Tove
Department of Animal Industry
North Carolina State College
Raleigh, North Carolina

Subject: CONTRACT NO. AT-(40-1)-1324

Dear Dr. Tove:

This is to advise you that we have initiated action for the preparation of a modification to Contract No. AT-(40-1)-1324 to extend the period to June 30, 1956, with the Commission contributing $6,618.00 to the total cost of $20,314.00. A copy of the budget to be included in the modification is enclosed for your information. The modification will be forwarded to you for signature as soon as it is complete.

Your cooperation is appreciated.

Very truly yours,

C. S. Shoup

C. S. Shoup
Chief, Biology Branch
Research and Medicine Division

Enclosure:
Budget

CC: J. G. Vann, North Carolina

Rounsaville:lr

Budget For Contract No. AT-(40-1)-1324

For Period 7/1/55 - 6/30/56

Salaries and Wages:		$11,625.00
Dr. S. B. Tove (½ time)	$3,125.00	
Other Faculty	3,000.00	
Research Assistants, Technicians, Animal Caretakers, Misc. Labor, Etc.	5,500.00	
Supplies:		1,100.00
Statistical Service:		1,250.00
Overhead: (45.36% of direct charges)		6,339.00
		$20,314.00

The Commission's contribution to the above budget will be $6,618.00.

Encl.

UNITED STATES
ATOMIC ENERGY COMMISSION

In Reply Refer To:
ADC:JN

Oak Ridge, Tennessee
May 26, 1955

North Carolina State College
Department of Animal Nutrition
Raleigh, North Carolina

Attention: Dr. Samuel B. Tove

Subject: MODIFICATION NO. 4 TO CONTRACT NO. AT-(40-1)-1324

Gentlemen:

Enclosed, duly signed on behalf of the Commission, are three copies of proposed Modification No. 4 to the subject contract which provides the program and budget for the fifth period of performance beginning July 1, 1955 and ending June 30, 1956. Also enclosed is a Notice of Research Project form which is to be used in submitting your summary statement.

If the modification, as submitted, is satisfactory to you it is requested that you sign the copies thereof in the space provided for the Senior Investigator and have the copies signed by the proper official of the College, returning one copy to this office as soon as possible. The two remaining copies are for your files. The summary statement should be returned along with the signed copy of the modification prior to June 30, 1955.

Very truly yours,

R. G. Humphries
Acting Director
Contract Division
Oak Ridge Operations

Enclosures:
Mod. 4 (in trip.)
Notice of Res. Project form

Nicholson:jn

Samuel
B

NORTH CAROLINA STATE COLLEGE
OF THE
UNIVERSITY OF NORTH CAROLINA
RALEIGH

DEPARTMENT
OF
CHEMICAL ENGINEERING

April 12, 1956

Mr. E. V. McGarry, Asst. Chief
Program Analysis Branch
Division of Biology and Medicine
U. S. Atomic Energy Commission
Washington 25, D. C.

Dear Mr. McGarry:

COPY

By means of the enclosed Section 3 information, and other information provided in our letter of December 23, 1955, we make application for discount approval for a project under the supervision of Dr. Samuel B. Tove.

The project is identified by us as Project 7. It has been in progress for some time on this campus, with AEC support, and involving not a few people.

Does this discount apply to processed material, such as C-14 labeled compounds sold by Tracerlab Incorporated, Boston, Massachusetts? If so, on what figure is the discount based?

Very truly yours,

F. Philips Pike, Chairman
Committee on Safety and Health
for the Nuclear Reactor and
Radioisotopes

FPP/c

cc: S. B. Tove

SECTION 3 INFORMATION FOR AEC FORM 372

APPLICATION FOR RADIOISOTOPE RESEARCH SUPPORT

3 Purpose of Research

(a) Carbon 14 labeled compounds are being used to study how the metabolism of lipids are affected by the composition of the diet. This project has been supported in part by AEC Contract AT-(40-1)-1324 since 1951.

(b) Animals and animal facilities are located in 317 Polk Hall, and equipment for chemical fractionation and counting are located in 317 and 318 Polk Hall.

(c) Has been in charge of the project since its inception.

(d) No work involving humans will be done.

Approved as Project 7

F. Philips Pike

F. Philips Pike, Chairman
Committee on Safety and Health
for the Nuclear Reactor and
Radioisotopes
North Carolina State College

Samuel B. Tove

Prof. Samuel B. Tove
317 Polk Hall
N. C. State College
Date: April 12, 1956

6-26-56

NOTICE OF RESEARCH PROJECT

Bio-Sciences Information Exchange
Smithsonian Institution

Supporting Agency: United States Atomic Energy Commission

Title: The effect of composition of the diet on lipide metabolism using C-14.

Personnel:

S. B. Tove, Department of Animal Industry, Research Associate Professor
H. L. Lucas, Department of Experimental Statistics, Professor
C. H. Wise, Department of Animal Industry, Professro
F. W. Sherwood, Department of Animal Industry, Professor
F. H. Smith, Department of Animal Industry, Associate Professor
N. Underwood, Department of Physics, Professor

Name and Address of Institution:

North Carolina State College, Raleigh, North Carolina

Summary:

Continue the study of the cyclic nature of the turnover curves of expired carbon dioxide using as tracers acetate and other metabolites labeled as though they were metabolically derived from acetate. Attempts will be made to express these curves as a series of exponentials.

To repeat a study of the effects of a linoleic acid deficiency on the turnover curves of the various fatty acids and lipides.

To establish the nature of the turnover of certain liver and blood compounds when all of the data of the specific activity-time curves come from the same animal.

NORTH CAROLINA STATE COLLEGE
SCHOOL OF AGRICULTURE
RALEIGH

Department of Animal Industry

June 26, 1956

MEMORANDUM TO: Dr. J. W. Pou

Three signed copies of the current A.E.C. contract are enclosed. They have to be signed by an officer of the College (I suppose Vann) and only 1 needs to be returned to the A.E.C., the other two are for the files here.

I also am enclosing a copy of the letter to me from Moore. You will note that he requests that a wire be sent notifying them as to the date, prior to June 30, the contract is signed and mailed. We had some trouble in this respect some years ago and as we discussed yesterday, we had best ask Murry or the secretary to handle this.

With respect to the budget, I am enclosing a copy of the telegram I received from Oak Ridge and also two copies of my reply.

You will recall that they turned down our request for an automatic flow counter ($3,400.) but gave us a total of $2,526. for the first period (July 1, 1956, to October 31, 1956). The total budget was not changed in the interest of expediency; therefore, the $3,400. equipment item was transferred to our side of the budget. You will also recall that I phoned Shoup on this very point and he said that even though the $3,400. was in equipment we were not committed to spend it there.

You will further note that the budget in the contract is only the total budget and does not show the breakdown by A.E.C. and by State College, but that the $3,400. equipment item is included.

Had there been time, we would have resubmitted the following budget:

	A.E.C. Support	N.C.S. Support	Total
Labor	$1,650.00	$2,252.25	$3,902.25
Supplies	231.00	120.00	351.00
Equipment	252.00	- - - -	252.00
Statistical Service	- - - -	412.50	412.50
Total Working Budget	$2,133.00	$2,784.75	$4,917.75
Overhead	393.00	1,837.69	2,230.69
Total	$2,526.00	$4,622.44	$7,148.44

Therefore, since we did not get the $3,400. for the piece of equipment, I do not think that the Business Office has any right to collect the overhead for it. We did get $252. extra and I think it fair that the Business Office receive the 8% of this or $20.

Samuel B. Tove
Research Associate Professor

SBT/ctw

UNITED STATES
ATOMIC ENERGY COMMISSION

In Reply Refer To:
ACC:JN

Oak Ridge, Tennessee
June 22, 1956

North Carolina State College
Department of Animal Nutrition
Raleigh, North Carolina

Attention: Dr. S. B. Tove

Subject: MODIFICATION NO. 5 TO CONTRACT NO. AT-(40-1)-1324

Gentlemen:

Enclosed, duly signed on behalf of the Commission, are three copies of Modification No. 5 to the subject contract which provides the program and budget for the sixth period of performance beginning July 1, 1956 and ending October 31, 1956. Also enclosed is a Notice of Research Project form which is to be used in submitting your summary statement.

If the modification, as submitted, is satisfactory to you it is requested that you sign the enclosed copies in the space provided for the Senior Investigator and have the copies signed by the proper official of the College, returning one copy to this office. The two remaining copies are for your retention. The summary statement should be returned along with the signed copy of the modification. It is requested that you advise this office by wire, prior to June 30, 1956, the date the modification is signed and mailed.

Very truly yours,

John R. Moore
Director
Contract Division
Oak Ridge Operations

Enclosures:
Mod. 5 (in trip.)
Res. Project form

UNITED STATES
ATOMIC ENERGY COMMISSION

In Reply
Refer To: ORS:JER

Oak Ridge, Tennessee
June 12, 1956

Dr. S. B. Tove
Department of Animal Nutrition
North Carolina State College
Raleigh, North Carolina

Subject: CONTRACT NO. AT-(40-1)-1324

Dear Dr. Tove:

This is to advise you that we have received notification from Washington to extend the period under Contract No. AT-(40-1)-1324 to October 31, 1957. However, the notification only authorized the Commission's contribution for the period July 1, 1956, to October 31, 1956, in the amount of $2,526. Therefore, we have initiated action for the preparation of a modification to extend the period to October 31, 1956, with the Commission contributing $2,526 in new funds to the total cost of $11,724.37.

The modification will also contain the necessary provisions to extend the period to October 31, 1957, and will include an outline of the total-cost budget for the period beginning November 1, 1956, with the Commission contributing $6,834 to the total cost of $20,604.78. However, an additional stipulation is being included to indicate that all provisions for the twelve-month period are subject to further written notification by the Commission. This notification will be forthcoming as soon as we receive an additional allocation of funds, which should be shortly after July 1.

Copies of the budgets to be included in the modification are enclosed for your information. The modification will be forwarded to you for signature as soon as it is complete. You should sign the document, obtain the signature of the appropriate official of the College and return it as soon as possible.

Your cooperation is appreciated.

Very truly yours,

C. S. Shoup

C. S. Shoup
Chief, Biology Branch
Research and Development Division

Enclosure:
Budgets (2)

cc: J. G. Vann, North Carolina, w/enclosure

BUDGET FOR CONTRACT NO. AT-(40-1)-1324
FOR PERIOD 11-1-56 - 10-31-57

Salaries and Wages:		$11,825.00
Dr. S. B. Tove (½ time)	$3,125.00	
Other Faculty	3,000.00	
Research Assistants, Technicians, Animal Caretakers, etc.	5,700.00	
Supplies:		1,100.00
Statistical Service:		1,250.00
Overhead (45.36% of direct charges):		6,429.78
TOTAL:		$20,604.78

The Commission's contribution to the above budget will be $6,834.

Copy of telegram to: June 11, 1956

Dr. C. S. Shoup, Chief
Biology Branch
Research and Development Division
U. S. Atomic Energy Commission
Oak Ridge, Tennessee

North Carolina State College will accept $2526 as AEC contribution to total cost of $11,724.37 for first 4 months of contract #AT-(40-1)-1324.

S. B. Tove

File AEC

NORTH CAROLINA STATE COLLEGE

SCHOOL OF AGRICULTURE • RALEIGH, N. C.

DEPARTMENT OF CHEMISTRY

July 2, 1956

COPY

Mr. W. L. Fleming
Purchasing Department
Campus

Dear Mr. Fleming:

This is to notify you that I have today received from the Atomic Energy Commission the discount certificate issued to North Carolina State College for purchase of radioisotopes. I am enclosing an official copy to you and one to Mr. David Lintz, Safety Officer. The certificate carries the number BM 57-114 and expires June 30, 1957. The certificate authorizes the purchase of radioisotopes at 20% of the AEC established price.

I am asking Mr. Lintz to certify requisitions as approved for discount and all requisitions should carry his initial before processing by your office.

We may use this certificate only for persons specifically authorized. As of this moment specific authorization has been obtained only for Dr. Gennard Matrone and Dr. Samuel Tove. The Safety Committee is prepared to obtain specific authorization for approved individuals and projects on request and without delay.

If there are questions, please call.

Sincerely,

Walter J. Peterson

Walter J. Peterson
Chairman, Committee on Safety
and Health for the Nuclear Reactor
and Radioisotopes

pp

Enclosure

cc: Mr. J. G. Vann
Dean J. H. Lampe
Dr. J. W. Pou
Dr. George Wise
Members of Safety and Health Committee
Mr. David Lintz
Dr. J. W. Fitts
Dr. G. Matrone
Dr. Sam Tove

NORTH CAROLINA STATE COLLEGE
SCHOOL OF AGRICULTURE
RALEIGH

Department of Animal Industry

March 25, 1957

MEMORANDUM TO: The Committee on Safety and Health for the Nuclear Reactor and Radioisotopes.

We have considered the recommendations of the Committee in the letter of October 30 and would like to modify the proposals made in our previous memorandum. The modifications are suggested in the interest of confinement of the isotope work and at the same time permitting the non-isotope portion of the research program to proceed with a minimum of inconvenience and time. The following are the suggestions:

1. That only Room 317 be designated as a "hot" laboratory. Room 318 will not be a "hot" laboratory and only non-radioactive work will be conducted in this laboratory. The facilities in Room 317 will be altered in accordance with the recommendations of the letter of October 30. It would seem possible to confine the isotope work to this room provided an additional laboratory bench is installed. However, because of the space limitations, Room 317 cannot be kept as a "hot" laboratory exclusively and some cold work will of necessity be conducted in this room. In order to provide for the maximum separation of "hot" and "cold" work within this room, the hood and immediate adjacent laboratory bench area will be designated as a "hot" area, and all glassware used in the isotope work will be kept separate and stored in this area. During the major portion of the year, it will be possible to confine the isotope work to this area. Furthermore, during the major portion of the year, the amount of activity present (other than source material) will be no more than 1 or 2 ucuries.

   When confinement of the "hot" work to the designated area is possible, it is suggested that the non-isotopic work be conducted in the "cold" area with cold glassware and equipment, and that normal laboratory procedures be permitted. It should be emphasized that the personnel conducting the isotope work will not be carrying out work in cold experiments at the same time. For those few times during the year when the scope of the isotope experiments will not permit confinement to the "hot" area, only "hot" work will be conducted in this laboratory. It is expected that only at these times will the level of activity be greater than the 1-2 ucurie level. At the end of this phase of the experiment when the great portion of the radioactivity is disposed of and the low level can be maintained, it is proposed that the "cold" section and any glassware and equipment in this area be decontaminated and monitered, and declared safe by the R.S.O. It is only then that "cold" work will be permitted in this area. It should be emphasized that these proposals are predicted on the assumption that an additional laboratory bench be provided in Room 317.

The other recommendations of the Safety Committee will be adopted.

The following is a list of C^{14} isotopes we have ordered. Since all of the experiments in which these were used were similar and no records were made of distribution, only rough approximations can be given. In all cases, approximately 90% of the activity was oxidized to carbon dioxide and disposed of via the hood. The remaining 10% was disposed of by combustion in the Power Plant furnaces. Less than 1% was disposed via the sewage system, and then only in high dilution.

Compound	Amt. Purchased	Date Purchased	Amount on Hand
Na Acetate	1 mc	1950	0
Na Acetate	1 mc	1951	0
Na Acetate	4 mc	1953	0
Stearic Acid	0.11 mc	1954	0.08 mc
Na Citrate	0.1 mc	1955	0.07 mc
$NaHCO_3$	0.5 mc	1955	0.1 mc
Na Acetate	1 mc	1956	0.9 mc
Na Propionate	0.1 mc	1956	0.08 mc

Samuel B. Tove
Research Associate Professor

SBT:msb

July 12, 1957

Mr. Paul B. Pearson
Chief, Biology Branch
Division of Biology and Medicine
U. S. Atomic Energy Commission
Washington 25, D. C.

Re: BMB:PBP

Dear Paul:

Enclosed are two copies of the summary of work that you requested in your recent letter. You will note I have interpreted your letter to mean brevity and general statements. I have not attempted to put in details as this is very difficult to put in laymen's terms as you no doubt know. I hope this is what you wanted. If not, let me know and there probably still will be time to get a revised statement in your hands.

Sincerely yours,

Samuel B. Tove
Research Associate Professor

SBT:msb

Enclosures

7-12-57

SUMMARY OF WORK

A.E.C. Contract No. AT-(40-1)-1324

Title: A Study of the Effect of Composition of the Diet on the Rate of Metabolism of Fatty Acids Using C^{14}.

Studies on the rate of metabolism of fat using radiocarbon as a tracer indicate that the metabolism of fat in an animal is a complex rapid cyclic process. Major cycles of build-up and breakdown occur at about 20-hour intervals, and minor cycles occur at much shorter intervals. If a similar tracer technique is applied to the expired carbon dioxide, major cycles appear at about 20-hour intervals and minor ones at about 2-hour intervals. These findings are part of an over-all effort aimed at a more intimate knowledge of the metabolic mechanism in animals with the ultimate goal of regulating the metabolic processes of man and animals in a predictable direction.

A.E.C. CONTRACT NO. AT-(40-1)-1324

Progress Report, Expenditure Statement, and Renewal Proposal

August 1, 1957

Prepared By: Sammy B Carr
Project Leader

Approved By: _____
Director, North Carolina
Agricultural Experiment Station

_____
Business Manager
North Carolina State College

Progress Report to

U. S. ATOMIC ENERGY COMMISSION
Research and Development Division
Post Office Box E
Oak Ridge, Tennessee

A. E. C. Contract #AT-(40-1)-1324

Contractor: North Carolina State College

Title: A Study of the Effect of Composition of the Diet on Lipids Metabolism Using C^{14}.

This report covers the period from April 1, 1956 to July 1, 1957. During this period studies were conducted on the effect of a linoleic acid deficiency on lipids metabolism and on the cyclic nature of specific activity time curves of expired carbon dioxide.

Linoleic Acid Experiment

A study of the effect of a linoleic acid deficiency on lipids metabolism was repeated. As in the previous experiment, two groups of rats were given a casein-sucrose purified diet. One of the groups (linoleic acid deficient) received no linoleic acid, whereas the second (control group) received 0.1 gm. methyl-linoleate per day. Feed consumption was adjusted by the paired feeding procedure. Six replications comprised the total experiment. After 23 to 25 weeks on this regime each animal was given 10 μc of carboxyl-labled acetate parenterally and a pair of rats was sacrificed 1, 2, 4, 6, 9 or 12 hours after the injection. Unlike the previous experiment in which only the liver lipides were sampled, the liver lipids, blood lipides, carcass lipides, fat-free liver, fat-free blood and fat-free carcass were assayed for radioactivity. In an attempt to account for all of the C^{14} injected, the carbon dioxide expired between the time of injection and sacrifice of each animal was collected and counted. Furthermore, the carbon dioxide was collected in two portions, that expired during the first hour and that expired between the first hour and the end of the experimental period.

The results of this experiment, shown in Figure 1, are similar to the results obtained in the hyperthyroid experiment conducted in 1955-1956 in which the specific activity time curves usually contained two peaks. Although the

presence of two peaks in the specific activity time curves is not as obvious as in the hyperthyroid experiments, they are apparent. It should be emphasized that each point on these curves represents the geometric mean of 6 animals and that this mathematical operation tends to "damp out" the peaks. If the data from the individual replications are graphed, the evidence of the peaks is much more striking. As in the thyroxine experiment (see 1955-1956 report), the peaks are much less apparent in samples of wide heterogenous composition, such as fat-free carcass. Attempts to account completely for the total C^{14} given each animal were not successful because of the errors associated with accurately determining the majority of the counts in the expired carbon dioxide. Nevertheless, plotting the data as percent of the total counts recovered (Figure 1) results in essentially the same turnover curves as those obtained by plotting the specific activity.

If the turnover curves of the two dietary treatment groups are compared visually, it would seem that a linoleic acid deficiency did not influence the metabolism of either acetate or its subsequent metabolites. An analysis of variants carried out on the data confirmed the lack of significant treatment effect. However, the high degree of animal variation associated with the cyclic nature of the turnover curves may preclude detection of a linoleic acid effect on metabolism.

Carbon Dioxide Specific-Activity Time Curves

Studies of the specific-activity time changes of expired carbon dioxide following the injection of radioacetate were continued from 1955-1956. In this study rats were given intraperitoneally 10 μc of carboxyl-labled acetate and immediately placed into glass jars and given food and water ad libitum. The jar lids were sealed with plastic tape and carbon dioxide-free air was forced through the jars and then through alkali to trap the expired carbon dioxide. The alkali traps were changed at 15-minute intervals. Barium chloride was added to an aliquot of each alkali sample, and the barium carbonate was filtered, washed, dried and counted.

In the previous experiments carbon dioxide collections were made over a period of 25 hours and carboxyl-labled acetate was the only compound studied. In the experiments conducted this year, the collection time was extended to 50 hours and 1-5 labled citrate, labled bicarbonate as well as carboxyl-labled acetate were used. Two rats received acetate, two received bicarbonate and three received citrate. The animals were observed during the experimental

period and their activity recorded every 15 minutes as either rest, active or eating.

The specific-activity time curves of one of the acetate animals and one of the citrate animals are shown in Figures 2 and 3, respectively. Five point moving averages of the individual points were plotted to smooth out minor fluctuations. Striking similarity between these two curves is evident on inspection, and this similarity carries through with the other five animals. Furthermore, the earlier portions of the 50-hour specific-activity time curves are in excellent agreement with the shorter specific-activity time curves obtained the past year.

Inspection of the curves reveals the presence of major cycles at about 20 hours intervals. Evidence of minor cycles at about 2 hour intervals also are visible. The minor cycles can be seen more readily by plotting the change in specific activity against time. This type of plot for the two curves of Figures 2 and 3 is shown in Figure 4. The minor cycles have been classified as to time of peak in the curves of change in specific activity with time. Although occasionally a peak is missing, when the peaks are present the mean variation in time of peaks between animals is about $\pm$ 0.75 hours.

From a comparison of the activity record with the change in specific activity time curves, it can be concluded that the time of peak is independent of animal activity or food consumption.

Results from experiments reported in this communication establish more firmly the cyclic nature of metabolism in an intact animal. Although one might attempt to ascribe these cycles to random variation, the consistency with which they occur and the regularity of their period makes this hypothesis very unlikely. Perhaps the most surprising observation is the similarity of the specific-activity time curves obtained with different compounds. It had been anticipated that by using different compounds some estimate of the rate of metabolism over a relatively narrow portion of the metabolic map might be obtained. In fact, the lack of difference is indicative that the cycles result from metabolic fluctuations over broad areas of metabolism.

Considerable time and effort was spent in an attempt to fit the carbon dioxide specific activity time data to a multi-exponential equation. Unfortunately, this was unsuccessful. The probable reason for this is that not

only was the model used of insufficient complexity, but also the present knowledge of statistical methods for fitting non-linear equations is inadequate. Nevertheless, work in this area is continuing.

Yeast Studies

In an effort to determine whether or not cycles would be found in the specific-activity time curve of expired carbon dioxide from a single-celled organism, two experiments were conducted with baker's yeast. A culture of yeast, isolated from commercial baker's yeast, was grown under aerobic conditions on a medium containing glucose, ammonium phosphate and corn steep liquor. Sixteen hours after inoculation, the cells were harvested by centrifugation, washed twice with isotonic saline and suspended in phosphate buffer at pH 6.8. The yeast suspension was aerated and the expired carbon dioxide was collected at 5 minute intervals for 12 hours after the addition of 1 μc of carboxyl-labled acetate. Cycles were found in the yeast carbon dioxide curves but only under conditions of endogenous metabolism. When glucose was present in the phosphate buffer, the cycles were not found. It should be emphasized that these results are preliminary and that the experiments must be repeated before these observations may be established.

Turnover of Compounds From a Single Animal

It had been anticipated that specific activity time curves for blood and liver compounds from a single animal (pig or dog) would be obtained. However, the inadequacy of available laboratory facilities from the radiological safety standpoint prevented the conduction of this study. It is expected that more adequate facilities will become available in time, and this phase of the program will be postponed until these facilities are available.

Publications

The Cyclic Nature of Turnover Curves. Federation Proceedings, 16:263, 1957.

Two manuscripts are in preparation: (1) the cyclic nature of turnover curves in tissue components and (2) the cyclic nature of turnover as detected by expired carbon dioxide.

Incidents

No incidents or accidents occurred during the current contract period.

Radioisotope Purchase Report

Quantity	Item	Price	Price C^{14}	Price Paid	Discount Certificate Savings
0.2 mc	Citric Acid 1,5 C^{14}	$135.00	$ 7.20	$129.25	$ 5.75
0.5 mc	Bicarbonate	50.00	18.00	35.60	14.40
1 mc	Acetate	85.00	36.00	56.20	28.80
		$270.00	$61.20	$221.05	$48.95

Expenditure of Funds

Budget of Available Funds:

	A.E.C. Support	N.C. State Support	Total
Balance of funds from 1955-1956 as of April 1, 1956:			
Salaries and Labor	$ 762.09	$ 1,706.25	$ 2,468.34
Supplies	194.97	-	194.97
Statistical Service	-	312.50	312.50
Overhead	279.50	1,305.26	1,584.76
Total	$1,236.56	$3,324.01	$4,560.57
Funds available July 1, 1956 to October 31, 1956:			
Salaries and Labor	$1,650.00	$2,252.25	$ 3,902.25
Supplies	231.00	120.00	351.00
Equipment	-	3,400.00	3,400.00
Statistical Service	-	412.50	412.50
Overhead	645.26	3,013.36	3,658.62
Total	$2,526.26	$9,198.11	$11,724.37
Funds available November 1, 1956 to October 31, 1957:			
Salaries and Labor	$5,000.00	$ 6,825.00	$11,825.00
Supplies	700.00	400.00	1,100.00
Statistical Service	-	1,250.00	1,250.00
Overhead	1,134.00	5,295.78	6,429.78
Total	$6,834.00	$13,770.78	$20,604.78

Funds Spent:

	A.E.C. Support	N. C. State Support	Total
April 1, 1956 to June 30, 1956:			
Salaries and Labor	$ 762.09	$ 1,706.25	$ 2,468.34
Supplies	194.97	-	194.97
Statistical Service	-	312.50	312.50
Overhead	279.50	1,305.26	1,584.76
Total	$1,236.56	$3,324.01	$4,560.57
July 1, 1956 to October 31, 1956:			
Salaries and Labor	$1,650.00	$ 2,252.25	$ 3,902.25
Supplies	231.00	120.00	351.00
Equipment	-	3,400.00	3,400.00
Statistical Service	-	412.50	412.50
Overhead	645.26	3,013.36	3,658.62
Total	$2,526.26	$9,198.11	$11,724.37
November 1, 1956 to July 31, 1957:			
Salaries and Labor	$4,000.00	$ 5,118.75	$ 9,118.75
Supplies	475.59	400.00	875.59
Statistical Service	-	937.50	937.50
Overhead	850.50	3,971.84	4,822.34
Total	$5,326.09	$10,428.09	$15,754.18
Funds anticipated to be spent August 1, 1957 to October 31, 1957:			
Salaries and Labor	$1,000.00	$1,706.25	$2,706.25
Supplies	224.41	-	224.41
Statistical Service	-	312.50	312.50
Overhead	283.50	1,323.94	1,607.44
Total	$1,507.91	$3,342.69	$4,850.60

Request for Renewal

of

A.E.C. Contract #AT-(40-1)-1324

Contractor: North Carolina State College

Title: A Study of the Effect of Composition of the Diet on Lipide Metabolism Using C^{14}.

Status of the Project:

The results of this project during the past three years establish that turnover curves of tissue components from animals are cyclic in nature. Major cycles occurring at about 15- to 20-hour intervals have been observed for hepatic fatty acids and expired carbon dioxide. Superimposed on the major cycles are minor ones of much shorter duration. It is clear that the presence of the cycles greatly complicates the problems not only of estimating metabolic rates of tissue metabolites in intact animals but also of ascertaining how these rates are affected by dietary or by environmental variables. Moreover, an understanding of the origin of the cycles is a fundamental requirement before turnover curves can be interpreted with confidence.

A priori, one would expect the specific-activity-time curves of expired carbon dioxide obtained following the injection of carboxyl-labled acetate, 1,5-labled citrate, and labled bicarbonate to differ. Such was not the case, probably because the cycles result from fluctuations in the metabolism of one or more of the larger metabolic systems common to all three compounds. Furthermore, since no correlation has been obtained between the time of the cycles and either the physical activity of the animal or ingestion of food, it would seem that the cycles are an inherent feature of animal metabolism. It would also appear that because of the simplicity of the experimental apparatus and the high degree of reproducibility encountered, the use of specific-activity-time curves of the expired carbon dioxide as an indirect method of estimating metabolic rates in intact animals warrants further investigation.

With respect to the origin of the cycles, we have considered four possibilities:

(1) There may be several metabolic pools of similar chemical composition that, although they may be in equilibrium with each other, have different rates of metabolism. These different pools may reside in different tissues within an animal, different cells within a single tissue or even different

parts of a single cell. Thus, even though a pure compound may be isolated, it would really represent the mean of two or more metabolic pools. Should the rates of metabolism of the individual pools be sufficiently different, the mean specific-activity-time curve would contain cycles.

(2) Similarly, the specific-activity-time curve for a common product arising by two or more alternate metabolic pathways would show cycles if the rates of metabolism of the alternate pathways were sufficiently different.

(3) Time-delay reactions in recycling of metabolites might occur. This can be described best by referring to the work of Stetten and Stetten (J. Biol. Chem., 213:723, 1955). These investigators have shown that shortly after the injection of glucose C^{14} the peripheral tiers of liver glycogen molecules had a higher specific activity than the center tiers. As time went on, the radioactivity of the center tiers rose, while that of the outer tiers fell, until finally the center tiers had a higher specific activity than the outer tiers. Thus, the glucose molecules move in a loop from the periphery of the glycogen, to the center and then out. The specific activity of the carbon dioxide from such animals would be expected to show cycles. It would be high initially, decrease as the glucose was in the center of the glycogen molecule and then rise again as the center tiers were oxidized. Tolbert et al. (U.C.R.L. 2941, 1955) did obtain a cycle in the specific-activity-time curve of expired carbon dioxide following the injection of radioglucose. One can speculate that similar events might occur with any large molecule not in true solution in a cell.

(4) The fourth concept involves the idea that metabolism is basically a discontinuous process. In other words, one metabolic system may function at an accelerated rate for a given period and then function at a reduced rate in the next period. At the same time, a second system follows the reverse time pattern. The specific-activity-time curves of products resulting from these systems would clearly be cyclic. This example is merely one of several possibilities that fall into this category. Among the others could be death, lysis and resynthesis of cells, and a process of alternate peeling off and laying on layers of some substance out of solution.

Proposed Work:

Studies on the specific-activity-time curves of expired carbon dioxide following the injections of different radio-compounds will be continued.

The compounds tested thus far are all in rapid equilibrium with the citric-acid cycle. Other compounds more distantly removed from the citric-acid cycle, such as glucose, glycine and higher fatty acids, will be tested. It is hoped that a comparison of the specific-activity-time curves obtained will yield information useful in elucidating the origin of the cycles.

Preliminary results obtained with aerated yeast suspensions indicate that the cycles also are present in expired carbon dioxide specific-activity-time curves of unicellular organisms. Furthermore, the cycles are observed only under conditions of endogenous metabolism and are not present when the cells are metabolizing exogenous glucose. If these findings can be substantiated by further experiments, an extremely useful system for studying the origin of the cycles is at hand; first because the absence or presence of the cycles can be controlled by the addition or removal of exogenous glucose, and second because frequent samples of the cells of a single uniform population can be obtained simultaneously with expired carbon dioxide.

The studies on the specific-activity-time curves of expired carbon dioxide from yeast will be repeated and extended to other unicellular organisms, such as bacteria and Ascites tumor cells. Should the cycles be present, samples of the cell populations will be taken at frequent intervals as the expired carbon dioxide turnover curves are obtained. These samples will then be fractionated into volatile material, carbohydrate, lipids and protein fractions, and their specific activity determined. The turnover curves of the isolated fractions will be compared with each other and with that of the expired carbon dioxide in an effort to obtain information bearing on the origin of the cycles. In addition, fractionation of the cell particulate bodies by differential centrifugation will be carried out to compare the turnover of the particulate fractions with that of the expired carbon dioxide.

Summary of Proposed Work for 1957-1958

(1) Continue the study on the cyclic nature of the specific-activity-time curves of expired carbon dioxide from rats using various C^{14} compounds, such as glucose, glycine and higher fatty acids.

(2) Continue and expand the study on the specific-activity-time curves of expired carbon dioxide from unicellular organisms, such as yeast, bacteria and Ascites tumor cells.

(3) Determine the nature of the specific-activity-time curves of various fractions of the unicellular organisms and compare these curves with the specific-activity-time curve of expired carbon dioxide.

Proposed Total Cost Budget

November 1, 1957 to October 31, 1958

	A.E.C. Support	N.C. State Support	Total
Salaries and Labor*			
Full-time technician	$ 3,550.00	-	$ 3,550.00
Half-time graduate student	2,000.00	-	2,000.00
S.B.Tove, Leader, 1/2 time	-	$ 4,000.00	4,000.00
Other personnel as listed in original project	-	3,330.00	3,330.00
Animal caretakers and misc. labor	-	770.00	770.00
Supplies	700.00	1,000.00	1,700.00
Equipment**	3,700.00	1,000.00	4,700.00
Statistical Service	-	1,500.00	1,500.00
Total working budget	$9,950.00	$11,600.00	$21,550.00
Overhead (41.03% of personal service. A.E.C. portion 8%, N.C.State portion 33.03%.	1,092.00	4,500.00	5,600.00
	$11,042.00	$16,108.00	$27,150.00

*The State of North Carolina has granted an 11% cost of living increase to all personnel; a similar increase is included for the personnel under this contract.

**The progress of the studies engaged in under this contract has been seriously hampered by lack of adequate counting facilities. During the past year more than 7,000 samples were counted, and the number will greatly increase with the proposed work. Therefore, it is most urgently requested that the additional funds required to obtain this counting equipment be granted.

November 8, 1957

Dr. C. S. Shoup
Chief, Biology Branch
Research and Development Division
United States Atomic Energy Commission
Oak Ridge, Tennessee

Dear Dr. Shoup:

In response to your letter of November 1, we will accept the contribution by the Atomic Energy Commission of $9,500 to a total cost of $27,150 for Contract No. AT-(40-1)-1324.

Very truly yours,

Samuel B. Tove
Research Associate Professor

SBT:msb

Copy to:
Dr. H. A. Stewart

File

UNITED STATES
ATOMIC ENERGY COMMISSION

IN REPLY REFER TO:
ORS:JDB

Oak Ridge, Tennessee
November 1, 1957

Dr. Samuel B. Tove
Department of Animal Nutrition
North Carolina State College
Raleigh, North Carolina

Subject: CONTRACT NO. AT-(40-1)-1324

Dear Dr. Tove:

This is to advise you that we have received approval for the preparation of a modification to Contract No. AT-(40-1)-1324 to extend the period during which the work may be performed to October 31, 1958. However, the AEC's contribution was approved at a level of $9,500 rather than $11,042 as requested in the proposal.

We shall appreciate your advising us if the College is willing to accept the contribution of $9,500 to a total cost of $27,150. If the College is unable to contribute the additional cost, we shall appreciate receiving a revised budget based on the reduced contribution of the AEC.

Our Headquarters office has suggested that expenditures for permanent type equipment be kept to a minimum since this will be the terminal year for the project. Please have the information you submit endorsed by the appropriate administrative officials of the College.

Your cooperation in submitting the appropriate information as soon as possible will be appreciated.

Very truly yours,

C. S. Shoup

C. S. Shoup
Chief, Biology Branch
Research and Development Division

cc: J. G. Vann, North Carolina
C. L. Dunham, Washington

BUDGET FOR CONTRACT NO. AT-(40-1)-1324
FOR PERIOD 11-1-57 - 10-31-58

Salaries and Wages:		$13,650.00
Dr. S. B. Tove (½ time)	$4,000.00	
Other Faculty	3,330.00	
Research Assistant, Technicians	5,550.00	
Animal Caretaker	770.00	
Supplies:		1,700.00
Equipment:		4,700.00
Statistical Service:		1,500.00
Overhead (41.03% of Salaries and Wages):		5,600.00
TOTAL		$27,150.00

The AEC's contribution to the above budget will be $9,500.

UNITED STATES
ATOMIC ENERGY COMMISSION

IN REPLY REFER TO:
ORS:JDB

Oak Ridge, Tennessee
December 5, 1957

Dr. Samuel B. Tove
Department of Animal Nutrition
North Carolina State College
Raleigh, North Carolina

Subject: CONTRACT NO. AT-(40-1)-1324

Dear Dr. Tove:

This is to advise you that we have initiated action for the preparation of a modification to Contract No. AT-(40-1)-1324 to extend the period to October 31, 1958, with the AEC contributing $9,500 in new funds to the total cost of $27,150. A copy of the budget to be included in the modification is enclosed for your information. The modification will be forwarded to you for signature as soon as it is complete.

Your cooperation is appreciated.

Very truly yours,

C. S. Shoup

C. S. Shoup
Chief, Biology Branch
Research and Development Division

Enclosure:
Budget

cc: J. G. Vann, North Carolina, w/encl.

File

NORTH CAROLINA STATE COLLEGE

SCHOOL OF AGRICULTURE • RALEIGH, N. C.

OFFICE OF THE DEAN AND DIRECTORS

November 25, 1957

Dr. C. S. Shoup
Chief, Biology Branch
Research and Development Division
United States Atomic Energy Commission
Oak Ridge, Tennessee

Subject: CONTRACT NO. AT-(40-1)-1324

Dear Dr. Shoup:

This is to advise you that North Carolina State College will accept an AEC contribution of $9,500 rather than $11,042 as requested in the original 1958 proposal for Contract No. AT-(40-1)-1324. A copy of the revised cost budget on the reduced contribution of AEC, endorsed by Mr. J. G. Vann, Business Manager of the College, is enclosed.

We appreciate your interest in and contribution to the support of this research. We hope that this information will enable you to complete the contract for the work to be performed to October 31, 1958.

Very truly yours,

R. L. Lovvorn
Director of Research

RLL
/
rca

Encl: Revised cost budget

CC: J. G. Vann

S. B. Tove

Revised Total Cost Budget

November 1, 1957 to October 31, 1958

Contract No. AT-(40-1)-1324	A.E.C. Support	N. C. State Support	Total
Salaries and Labor			
Full-time technician	$3,550.00	$ - -	$3,550.00
Half-time graduate student	2,000.00	- -	2,000.00
S. B. Tove, Leader, 1/2 time	- -	4,000.00	4,000.00
Other personnel as listed in original project	- -	3,330.00	3,330.00
Animal caretakers and misc. labor	- -	770.00	770.00
Supplies	700.00	1,000.00	1,700.00
Equipment	2,546.00	2,154.00	4,700.00
Statistical Service	- -	1,500.00	1,500.00
Total working budget	$8,796.00	$12,754.00	$21,550.00
Overhead:			
41.03% of all salaries and wages		5,600.00	5,600.00
AEC-8% of working budget	704.00	(704.00)	
	$9,500.00	$17,650.00	$27,150.00

Financial provisions for this agreement approved

J. G. Vann
Business Manager

UNITED STATES
ATOMIC ENERGY COMMISSION

COPY

ORS:AMC

Oak Ridge, Tennessee

Subject: RENEWAL OF CONTRACT NO. AT-(40-1)-

Your attention is invited to Appendix "C" of the contract which provides that a renewal proposal and a progress report will be due
The renewal proposal and progress report may be submitted at any time after six months of performance under the current contract period. In any event, they should reach us no later than the prescribed date.

The information to be submitted is as follows:

1. Progress Report. Include information outlined in Appendix "C".

2. Expenditure Statement. Include a current expenditure statement for the present contract period. Information included should be as follows:

   A. Statement should be based on both the contributions of the institution and the AEC.

   B. The statement should reflect (1) the costs of the project to date, (2) an estimate of the costs for the remainder of the contract period, and (3) an estimate of the funds which will be available for financing the project during the proposed period of performance.

3. Renewal Proposal. Include the information as required in Appendix "C" of the contract. A budget should be included indicating the total costs to carry the project for the proposed period, based on the contributions of the institution as well as the funds requested from the AEC.

4. Ten-Percent Voucher. Include a voucher for the remaining ten percent of the agreed consideration as provided by Article III of the contract. This voucher will be held by us, pending approval for renewal of the contract, after which it will be paid or if contract is not renewed, it will be paid upon receipt of the complete scientific report.

5. Incident Report. Include information as required in attachment "A".

The progress report, expenditure statement, and renewal proposal should be endorsed by the proper administrative officials, and six copies of each, together with the voucher, should be submitted at the same time to:

U. S. Atomic Energy Commission
Research and Development Division
Post Office Box E
Oak Ridge, Tennessee

If you do not contemplate requesting renewal, we shall appreciate your advising us as soon as possible.

Your cooperation and assistance in this matter is appreciated.

Very truly yours,

(Signed) HERMAN M. ROTH

Herman M. Roth
Director
Research and Development Division

Enclosure:
Attachment "A" (1)

ATTACHMENT "A"

Incident Reports

AEC regulations require that reports be submitted on accidents, fires, and unusual operational incidents which occur in connection with work being performed under AEC supported contracts. Reports are required for the following types of incidents:

1. Serious Incidents

   Immediate reports by phone (Oak Ridge 5-8611, extension 4657) or teletype to Research and Development Division, U. S. Atomic Energy Commission, Post Office Box E, Oak Ridge, Tennessee, on fatal injury where death seems imminent; injury of five or more in one fire or accident; damage of $5,000 or more to Government property; nuclear radiation of personnel or chemical exposure, resulting in overexposure sufficiently serious to warrant hospitalization for observation or treatment; and other accidents or incidents of which immediate information is considered in the best interest of the program.

2. Fires, Explosions, Lightning, Windstorms, Floods, and Sprinkler Leakage

   Reports on industrial loss of $50 to $5,000 of Government property from these causes, together with any loss and damage to private property.

3. Other Property Damage Accidents

   Reports on other accidental property damage of $50 to $5,000 to Government property.

4. Personal Injuries

   Reports of personal injuries, including disability from occupational disease and nuclear radiation occurring in connection with work being performed under AEC supported contracts.

Reports on items 2, 3, and 4 may be included in the annual progress or final report. If no such incidents have occurred during the current contract period, please include a statement to this effect in your progress report or final report.

May 8, 1959

MEMORANDUM TO: Dr. R. L.Lovvorn, Director
N. C. Agricultural Experiment Station
Patterson Hall
Campus

FROM: George H. Wise, Head
Animal Nutrition Section
Department of Animal Industry

Subject: Progress Report and Expenditure Statement for A.E.C. Contract No. AT-(40-1)-1324.

Attached are eight copies of a report prepared for the U. S. Atomic Energy Commission by Dr. S. B. Tove. Please note that AEC support of the project was terminated October 31, 1958, but Dr. Tove is requesting that the contract be renewed on a no-cost basis to permit completion of several phases of the research still in progress.

If the report, including research progress and expenditure statement for the period of from July 1, 1957 to May 7, 1959, is acceptable, we should appreciate your signing, requesting Mr. Vann's signature of approval, and finally transmitting six approved copies to the following address:

U. S. Atomic Energy Commission
Research and Development Division
Post Office Box E
Oak Ridge, Tennessee

Attachments

Approved: _____
George Hyatt, Jr., Head
Department of Animal Industry

Project Leader

A.E.C. CONTRACT NO. AT-(40-1)-1324

Progress Report
and
Expenditure Statement

May 7, 1959

Prepared By: _____
Project Leader

Approved By: _____
Director, North Carolina
Agricultural Experiment Station

_____
Business Manager
North Carolina State College

Progress Report to

U. S. ATOMIC ENERGY COMMISSION
Research and Development Division
Post Office Box E
Oak Ridge, Tennessee

A. E. C. Contract #AT-(40-1)-1324

Contractor: North Carolina State College

Title: A Study of the Effect of Composition of the Diet on Lipide Metabolism Using C^{14}.

This report covers the period from July 1, 1957 to date.

Inasmuch as the contract with the Atomic Energy Commission was to terminate as of October 31, 1959, and since it was felt that support for work along the same lines would be unobtainable from sources outside the AEC, the direction of the work was shifted somewhat.

Studies had been conducted at this laboratory on the kinetics of depletion of linoleic acid from animal fat depots. In these studies high depot fat levels of linoleic acid were induced in mice by feeding safflower oil. The animals were then given a fat-free diet and the disappearance of linoleic acid from the depot fat followed. It was observed that there were at least two rates of decline, a rapid one and a relatively slow one. One of the questions to be answered was whether the slow rate was specific for linoleic acid or whether it reflected a lipide pool of slow metabolism.

In an attempt to determine this, two experiments with C^{14}-labeled fatty acids were conducted. In the first experiment carboxyl-labeled linoleic acid and palmitic acid were used and in the second, carboxyl-labeled stearic acid as well as linoleic and palmitic were used. In these experiments the C^{14}-labeled acids were fed for 2-3 weeks with a diet containing 15% safflower oil, after which a fat-free, C^{14}-free diet was fed for 3-6 weeks. The expired carbon dioxide was collected daily during the course of the entire experiments and counted. The results may be summarized as follows:

1. Despite the fact that the specific activity of the dietary linoleic was less than one-tenth that of the palmitic and stearic acids, the total counts expired from all acids as well as the specific activity of the expired carbon dioxide was remarkably similar.

2. The curves of the radioactivity of the expired carbon dioxide, after cessation of the C^{14} feeding, showed two slopes, indicating that there are at least two pools of lipides that are metabolized at different rates.

3. The data from the second experiment were adjusted statistically for daily food intake and the daily activity (as measured by weight of CO_2 expired). This treatment of the data resulted in greatly smoothing out the daily variations in the curves. Of greater significance, however, the statistical treatment showed that there was a specific increase in the $C^{14}O_2$ from linoleate associated with daily activity. This interesting observation is undergoing further study.

Incidents

No incidents or accidents occurred during the contract period covered by this report.

Request for renewal of contract: We request a renewal of the contract on a no-cost basis for the following year to complete the studies now in progress.

Expenditure of Funds

Budget of Available Funds:

	A.E.C. Support	N. C. State Support	Total
Balance of funds from 1955-1956 as of August 1, 1957:			
Salaries and labor	1,000.00	1,706.25	2,706.25
Supplies	224.41	--	224.41
Statistical Service	--	312.50	312.50
Overhead	283.50	1,323.94	1,607.44
	1,507.91	3,342.69	4,850.60
Funds available from November 1, 1957 to October 31, 1958:			
Salaries and labor	5,550.00	8,100.00	13,650.00
Supplies	700.00	1,000.00	1,700.00
Equipment	2,158.00	2,542.00	4,700.00
Statistical Service	--	1,500.00	1,500.00
Overhead	1,092.00	4,508.00	5,600.00
	9,500.00	17,650.00	27,150.00

Funds Spent:

	A.E.C. Support	N. C. State Support	Total
August 1, 1957 to October 31, 1957:			
Salaries and labor	1,000.00	1,706.25	2,706.25
Supplies	224.41	--	224.41
Statistical Service	--	312.50	312.50
Overhead	283.50	1,323.94	1,607.44
	1,507.91	3,342.69	4,850.60
November 1, 1957 to October 31, 1958:			
Salaries and labor	5,550.00	8,100.00	13,650.00
Supplies	700.00	1,000.00	1,700.00
Equipment	2,158.00	2,542.00	4,700.00
Statistical Service	--	1,500.00	1,500.00
Overhead	1,092.00	4,508.00	5,600.00
	9,500.00	17,650.00	27,150.00

UNITED STATES
ATOMIC ENERGY COMMISSION

IN REPLY REFER TO:
ACC:ARB

Oak Ridge, Tennessee
July 1, 1959

North Carolina State College
School of Agriculture
Office of the Dean and Directors
Raleigh, North Carolina

Attention: Dr. H. A. Stewart, Assistant Director for Research

Subject: MODIFICATION NO. 7 TO CONTRACT NO. AT-(40-1)-1324

Gentlemen:

Enclosed, for your retention, is one duly signed copy of the subject modification.

Very truly yours,

ORIGINAL SIGNED BY
JOHN R. MOORE

John R. Moore
Director, Contract Division
Oak Ridge Operations

Enclosure:
Mod. 7

Contract No. AT-(40-1)-1324
North Carolina State College of Agriculture
and Engineering
Modification No. 7

SUPPLEMENTAL AGREEMENT

THIS SUPPLEMENTAL AGREEMENT, entered into this 30th day of June, 1959, by and between the UNITED STATES OF AMERICA (hereinafter called the "Government"), represented herein by the UNITED STATES ATOMIC ENERGY COMMISSION (hereinafter called the "Commission"), and NORTH CAROLINA STATE COLLEGE OF AGRICULTURE AND ENGINEERING (hereinafter called the "Contractor");

WITNESSETH THAT:

WHEREAS, the Government and the Contractor entered into Contract No. AT-(40-1)-1324, dated June 29, 1951, providing for a study of the effect of the composition of the diet on lipide metabolism; and

WHEREAS, the contract has been amended heretofore by Modifications Nos. 1 - 6; and

WHEREAS, the Commission desires to provide for an extension of the time during which the work under TITLE VIII of Appendix "A" may be conducted, without additional funds, and to effect certain other changes as are hereinafter more particularly described; and

WHEREAS, this Supplemental Agreement is authorized by and executed under the Atomic Energy Act of 1954;

NOW, THEREFORE, the parties hereto do mutually agree that said contract is hereby modified in the following particulars, but in no others:

1. The following new section "9." is added to Article II:

"9. The period during which the work under this contract may be conducted is hereby extended to October 31, 1959."

2. Subsection 2. a. of Article III is revised to read as follows:

"a. On or before the date of commencement of the work on the project described in Appendix "A", the Government shall pay to the Contractor upon submission by the Contractor of a proper voucher, 45 per cent of the agreed consideration."

3. In subsections 2. b. and 2. c. of Article III, delete the words "properly certified voucher" and substitute therefor the words "proper voucher."

IN WITNESS WHEREOF, the parties hereto have executed this Supplemental Agreement the day and year first above written.

UNITED STATES OF AMERICA

BY: UNITED STATES ATOMIC ENERGY COMMISSION

BY: Herman M. Roth

HERMAN M. ROTH
DIRECTOR
RESEARCH AND DEVELOPMENT DIVISION

(Contracting Officer)

WITNESSES:

H. A. Stewart

Raleigh, N.C.
(Address)

Mrs. Lewis Blackwood

Raleigh, N.C.
(Address)

NORTH CAROLINA STATE COLLEGE OF AGRICULTURE AND ENGINEERING

BY: J. V. Harris

TITLE: BUSINESS MANAGER

ACCEPTANCE BY SENIOR INVESTIGATOR

I have read the foregoing Supplemental Agreement and agree to be bound by the provisions of this document.

Herman B Bloch

Senior Investigator

NORTH CAROLINA STATE COLLEGE

SCHOOL OF AGRICULTURE • RALEIGH, N. C.

OFFICE OF THE DEAN AND DIRECTORS

June 26, 1959

COPY

U. S. Atomic Energy Commission
Oak Ridge, Tennessee

Attention: Director John R. Moore
Contract Division
Oak Ridge Operations

Reference: ACC:ARB

Subject: MODIFICATION NO. 7 TO CONTRACT NO. AT-(40-1)-1324

Gentlemen:

Enclosed are duplicate copies of Modification No. 7 to Contract No. AT-(40-1)-1324 properly signed and witnessed.

Very truly yours,

H. A. Stewart

H. A. Stewart
Assistant Director of Research

HAS:ks

Enc. 2

c.c. S. B. Tove
Enc. 2 - Original letter from AEC
Third copy of Modification No. 7

UNITED STATES
ATOMIC ENERGY COMMISSION

IN REPLY REFER TO:
ACC:ARB

Oak Ridge, Tennessee
June 24, 1959

North Carolina State College
School of Agriculture
Department of Animal Industry
Raleigh, North Carolina

Attention: Dr. Samuel B. Tove

Subject: MODIFICATION NO. 7 TO CONTRACT NO. AT-(40-1)-1324

Gentlemen:

Enclosed, in triplicate, is proposed Modification No. 7 to the subject contract which provides for an extension of the time, to October 31, 1959, during which the work under the contract may be conducted, without additional funds.

If the modification, as submitted, is satisfactory, will you kindly sign two of the enclosed copies in the space provided for the Senior Investigator, have such two copies signed by the proper official of the College, and return them to this office. The third enclosed copy is for your retention. After signature and dating on behalf of the Commission, one duly signed copy of the modification will be returned for your files.

Very truly yours,

John R. Moore
Director, Contract Division
Oak Ridge Operations

Enclosure:
Proposed Mod. 7 (in trip.)

CC: Div. of Biology & Medicine,
AEC Headquarters
C. S. Shoup

Contract No. AT-(40-1)-1324
North Carolina State College of Agriculture
and Engineering
Modification No. 7

SUPPLEMENTAL AGREEMENT

THIS SUPPLEMENTAL AGREEMENT, entered into this 26th day of June, 1959, by and between the UNITED STATES OF AMERICA (hereinafter called the "Government"), represented herein by the UNITED STATES ATOMIC ENERGY COMMISSION (hereinafter called the "Commission"), and NORTH CAROLINA STATE COLLEGE OF AGRICULTURE AND ENGINEERING (hereinafter called the "Contractor");

WITNESSETH THAT:

WHEREAS, the Government and the Contractor entered into Contract No. AT-(40-1)-1324, dated June 29, 1951, providing for a study of the effect of the composition of the diet on lipide metabolism; and

WHEREAS, the contract has been amended heretofore by Modifications Nos. 1 - 6; and

WHEREAS, the Commission desires to provide for an extension of the time during which the work under TITLE VIII of Appendix "A" may be conducted, without additional funds, and to effect certain other changes as are hereinafter more particularly described; and

WHEREAS, this Supplemental Agreement is authorized by and executed under the Atomic Energy Act of 1954;

NOW, THEREFORE, the parties hereto do mutually agree that said contract is hereby modified in the following particulars, but in no others:

1. The following new section "9." is added to Article II:

"9. The period during which the work under this contract may be conducted is hereby extended to October 31, 1959."

2. Subsection 2. a. of Article III is revised to read as follows:

"a. On or before the date of commencement of the work on the project described in Appendix "A", the Government shall pay to the Contractor upon submission by the Contractor of a proper voucher, 45 per cent of the agreed consideration."

3. In subsections 2. b. and 2. c. of Article III, delete the words "properly certified voucher" and substitute therefor the words "proper voucher."

IN WITNESS WHEREOF, the parties hereto have executed this Supplemental Agreement the day and year first above written.

UNITED STATES OF AMERICA

BY: UNITED STATES ATOMIC ENERGY COMMISSION

BY: _____

_____

(Contracting Officer)

WITNESSES:

H. A. Stewart

Raleigh, N.C.

(Address)

(Mrs) Louise Blackwood

Raleigh, N.C.

(Address)

NORTH CAROLINA STATE COLLEGE OF AGRICULTURE AND ENGINEERING

BY: _____

TITLE: BUSINESS MANAGER

ACCEPTANCE BY SENIOR INVESTIGATOR

I have read the foregoing Supplemental Agreement and agree to be bound by the provisions of this document.

_____

Senior Investigator

A.E.C. CONTRACT NO. AT-(40-1)-1324

Final Report

September 7, 1960

Prepared By: _____
Project Leader

Approved By: _____
Director, North Carolina
Agricultural Experiment Station

_____
Business Manager
North Carolina State College

Final Report to

U. S. ATOMIC ENERGY COMMISSION
Research and Development Division
Post Office Box E
Oak Ridge, Tennessee

A. E. C. Contract No. AT-(40-1)-1324

Contractor: North Carolina State College.

Title: A Study of the Effect of Composition of the Diet on Lipide Metabolism Using C^{14}.

This report covers the period from July 1,1951, to October 31, 1959.

Objectives:

When this project was initiated, it was hoped that lipide metabolism could be studied by investigating the effects of various nutritional factors (e.g., caloric intake, essential fatty acids, pantothenic acid, biotin) on the turnover of liver fatty acids. As the work progressed, the high degree of variability encountered in the data resulted in a shift of the objectives toward a more fundamental study of the nature of turnover curves.

Results:

In the initial phase of the project the effect of a deficiency of pantothenic acid, biotin, linoleic acid, and pyridoxine on the turnover of palmitic acid, stearic acid, and the unsaturated fatty acids was studied. In addition, the effects of caloric insufficiency and thyroxine feeding were also studied. In all of these experiments a single injection of carboxyl-labeled acetate was used as the tracer dose. In the first

experiments observations were taken at 12-hour intervals; and although the deficient groups were variable, the control groups clearly showed two turnover curves within 48 hours. The latter provided a basis for a paper entitled "Turnover of Palmitic, Stearic and Unsaturated Fatty Acids in Rat Liver", published in the Journal of Biological Chemistry (218: 275, 1956). In subsequent experiments the observations were taken at shorter time intervals.

The most characteristic feature of the data of all of these later experiments was the marked degree of variability between animals of the same experiment and between different experiments. This marked degree of variability was such that it was impossible to decide whether or not any of the nutritional variables under study had any effect on the metabolism of lipides. Thus the problem became a study of the variation; and as such, distinguishing animal variation of the usual random type from that which was not random but represented a phenomenon associated with metabolic turnover, became of paramount importance. It was considered that metabolic turnover might not be represented as a smooth curve but rather a curve consisting of a complex series of damped cycles. With such a situation highly variable data would be expected, for the variation would thus be composed of two components: (1) variability in the total amount of tracer that passes through a pool, and (2) variability in the rate of transfer from one pool to another, i.e., the degree with which metabolic cycles of different animals are in or out of phase. Clearly what is needed is a continuous record of the turnover of a single compound in one animal. Accordingly, the turnover of several compounds, as measured by expired carbon dioxide, was investigated.

In these studies, the specific activity time curves for expired carbon dioxide were constructed from observations taken at 15-minute intervals

over periods of from 20 to 50 hours. The tracer compounds injected were carboxyl-labeled acetate, 1-5 labeled citrate, and labeled bicarbonate. All of the curves obtained were similar, clearly showing the presence of damped cycles. Major cycles of about 20 hours duration were observed in good agreement with the previously observed binodal turnover curves of liver fatty acids. Superimposed on the major cycles were minor cycles showing some degree of regularity. During some of these experiments, observations on the activity of the animals were taken, but neither time of food ingestion nor animal activity could be correlated with the cycles.

Considerable time and effort were expended during the project on attempts to fit the data to multi-exponential equations. In all cases these attempts met with complete failure. The chief reason for this lay in the fact that statistical knowledge for the fitting of the data to non-linear equations was inadequate.

Discussion:

It is clear from these studies conducted under this contract that metabolic turnover curves are cyclic in nature. In speculating on mechanisms that would account for cyclic turnover curves, four possibilities have been considered:

1. There could be more than one metabolic pool for a given compound. These different pools might reside in different tissues within an animal, different cells within a given tissue, different parts of a single cell, or even in different parts of a large molecule. Thus, even though a pure compound was isolated, it would represent the mean of several parts.

Should the individual pools have metabolic rates sufficiently different, the mean specific activity time curves would contain cycles.

2. Similarly, cycles would occur in the specific activity time curve of a metabolite that arose from a common precursor over two or more alternate pathways with different metabolic rates.

3. A time delay in the recycling of a metabolite might occur. For example, Stetten and Stetten (J. Biol. Chem., 213: 723, 1955) found that shortly after the injection of labeled glucose, the peripheral tiers of liver glycogen were more highly labeled than the center tiers. As time went on, the radioactivity of the center tiers rose, while that of the peripheral tiers fell, until finally the center tiers of the glycogen had a higher specific activity than the outer tiers. Thus the glucose move in a loop from the periphery of the glycogen molecule to the center and then out. The specific activity of carbon dioxide from such animals would be high initially, decrease as the glucose was in the center of the glycogen molecule, and then increase as the glucose which occupied the center tiers was oxidized. Tolbert et al. (U.C.R.L., 2941, 1955) did obtain a cycle in the specific activity time curve of the expired carbon dioxide following the injection of radioglucose. It is not unreasonable that similar events would occur with any large molecule not in true solution in a cell.

4. That a specific activity time curve should be uniform is based on the assumption that metabolism flows continuously smoothly and evenly. It would seem logical that such an assumption is an oversimplification of the true picture of metabolism particularly in an intact animal. It is more likely metabolism is basically a more or less discontinuous process. As such, a given metabolic pathway may function at an accelerated rate for a given period and at a reduced rate for the next period. At the same time, a second system could follow a reverse time pattern. The specific activity time curves resulting from these systems would clearly be cyclic.

It was a great disappointment that no progress could be made in fitting the specific activity time data to exponential expressions of rate. It is hoped that with improvements in the estimation of non-linear equations, this goal will be achieved in the future.

Finally, despite the vast amount of work on the estimation of turnover rates, one can question what is measured in these investigations. In almost every instance (where sufficient observations were taken) these curves show evidence of cycles that were assumed to be random variation and hence were ignored. This does not mean to imply that genuine turnovers were not measured in these cases, but the difficulty arises in deciding which metabolic compartment is represented by the turnover values obtained.

Incidents:

No incidents or accidents occurred other than those of a minor nature during the contract period.

Termination:

With this report, work on A.E.C. contract No. AT-(40-1)-1324 will be terminated.

Expenditure of Funds:

The financial statement for the contract has been made previously, and no further expenditure of funds has been made since the last report.

A.E.C. CONTRACT NO. AT-(40-1)-1324

Final Report

September 7, 1960

Prepared By: _____
Project Leader

Approved By: _____
Director, North Carolina
Agricultural Experiment Station

_____
Business Manager
North Carolina State College

Final Report to

U. S. ATOMIC ENERGY COMMISSION
Research and Development Division
Post Office Box E
Oak Ridge, Tennessee

A. E. C. Contract No. AT-(40-1)-1324

Contractor: North Carolina State College.

Title: A Study of the Effect of Composition of the Diet on Lipide Metabolism Using C^{14}.

This report covers the period from July 1,1951, to October 31, 1959.

Objectives:

When this project was initiated, it was hoped that lipide metabolism could be studied by investigating the effects of various nutritional factors (e.g., caloric intake, essential fatty acids, pantothenic acid, biotin) on the turnover of liver fatty acids. As the work progressed, the high degree of variability encountered in the data resulted in a shift of the objectives toward a more fundamental study of the nature of turnover curves.

Results:

In the initial phase of the project the effect of a deficiency of pantothenic acid, biotin, linoleic acid, and pyridoxine on the turnover of palmitic acid, stearic acid, and the unsaturated fatty acids was studied. In addition, the effects of caloric insufficiency and thyroxine feeding were also studied. In all of these experiments a single injection of carboxyl-labeled acetate was used as the tracer dose. In the first

experiments observations were taken at 12-hour intervals; and although the deficient groups were variable, the control groups clearly showed two turnover curves within 48 hours. The latter provided a basis for a paper entitled "Turnover of Palmitic, Stearic and Unsaturated Fatty Acids in Rat Liver", published in the Journal of Biological Chemistry (218: 275, 1956). In subsequent experiments the observations were taken at shorter time intervals.

The most characteristic feature of the data of all of these later experiments was the marked degree of variability between animals of the same experiment and between different experiments. This marked degree of variability was such that it was impossible to decide whether or not any of the nutritional variables under study had any effect on the metabolism of lipides. Thus the problem became a study of the variation; and as such, distinguishing animal variation of the usual random type from that which was not random but represented a phenomenon associated with metabolic turnover, became of paramount importance. It was considered that metabolic turnover might not be represented as a smooth curve but rather a curve consisting of a complex series of damped cycles. With such a situation highly variable data would be expected, for the variation would thus be composed of two components: (1) variability in the total amount of tracer that passes through a pool, and (2) variability in the rate of transfer from one pool to another, i.e., the degree with which metabolic cycles of different animals are in or out of phase. Clearly what is needed is a continuous record of the turnover of a single compound in one animal. Accordingly, the turnover of several compounds, as measured by expired carbon dioxide, was investigated.

In these studies, the specific activity time curves for expired carbon dioxide were constructed from observations taken at 15-minute intervals

over periods of from 20 to 50 hours. The tracer compounds injected were carboxyl-labeled acetate, 1-5 labeled citrate, and labeled bicarbonate. All of the curves obtained were similar, clearly showing the presence of damped cycles. Major cycles of about 20 hours duration were observed in good agreement with the previously observed binodal turnover curves of liver fatty acids. Superimposed on the major cycles were minor cycles showing some degree of regularity. During some of these experiments, observations on the activity of the animals were taken, but neither time of food ingestion nor animal activity could be correlated with the cycles.

Considerable time and effort were expended during the project on attempts to fit the data to multi-exponential equations. In all cases these attempts met with complete failure. The chief reason for this lay in the fact that statistical knowledge for the fitting of the data to non-linear equations was inadequate.

Discussion:

It is clear from these studies conducted under this contract that metabolic turnover curves are cyclic in nature. In speculating on mechanisms that would account for cyclic turnover curves, four possibilities have been considered:

1. There could be more than one metabolic pool for a given compound. These different pools might reside in different tissues within an animal, different cells within a given tissue, different parts of a single cell, or even in different parts of a large molecule. Thus, even though a pure compound was isolated, it would represent the mean of several parts.

Should the individual pools have metabolic rates sufficiently different, the mean specific activity time curves would contain cycles.

2. Similarly, cycles would occur in the specific activity time curve of a metabolite that arose from a common precursor over two or more alternate pathways with different metabolic rates.

3. A time delay in the recycling of a metabolite might occur. For example, Stetten and Stetten (J. Biol. Chem., 213: 723, 1955) found that shortly after the injection of labeled glucose, the peripheral tiers of liver glycogen were more highly labeled than the center tiers. As time went on, the radioactivity of the center tiers rose, while that of the peripheral tiers fell, until finally the center tiers of the glycogen had a higher specific activity than the outer tiers. Thus the glucose move in a loop from the periphery of the glycogen molecule to the center and then out. The specific activity of carbon dioxide from such animals would be high initially, decrease as the glucose was in the center of the glycogen molecule, and then increase as the glucose which occupied the center tiers was oxidized. Tolbert et al. (U.C.R.L., 2941, 1955) did obtain a cycle in the specific activity time curve of the expired carbon dioxide following the injection of radioglucose. It is not unreasonable that similar events would occur with any large molecule not in true solution in a cell.

4. That a specific activity time curve should be uniform is based on the assumption that metabolism flows continuously smoothly and evenly. It would seem logical that such an assumption is an oversimplification of the true picture of metabolism particularly in an intact animal. It is more likely metabolism is basically a more or less discontinuous process. As such, a given metabolic pathway may function at an accelerated rate for a given period and at a reduced rate for the next period. At the same time, a second system could follow a reverse time pattern. The specific activity time curves resulting from these systems would clearly be cyclic.

It was a great disappointment that no progress could be made in fitting the specific activity time data to exponential expressions of rate. It is hoped that with improvements in the estimation of non-linear equations, this goal will be achieved in the future.

Finally, despite the vast amount of work on the estimation of turnover rates, one can question what is measured in these investigations. In almost every instance (where sufficient observations were taken) these curves show evidence of cycles that were assumed to be random variation and hence were ignored. This does not mean to imply that genuine turnovers were not measured in these cases, but the difficulty arises in deciding which metabolic compartment is represented by the turnover values obtained.

Incidents:

No incidents or accidents occurred other than those of a minor nature during the contract period.

Termination:

With this report, work on A.E.C. contract No. AT-(40-1)-1324 will be terminated.

Expenditure of Funds:

The financial statement for the contract has been made previously, and no further expenditure of funds has been made since the last report.

NORTH CAROLINA STATE COLLEGE
SCHOOL OF AGRICULTURE • RALEIGH, N. C.

OFFICE OF THE DEAN AND DIRECTORS

COPY

September 13, 1960

Reference: ORS:JDB

Dr. Herman M. Roth, Director
Research and Development Division
U. S. Atomic Energy Commission
Oak Ridge, Tennessee

Subject: Contract No. AT-(40-1)-1324

Dear Dr. Roth:

Enclosed are six copies of the final report under AEC Contract No. AT-(40-1)-1324, which expired on October 31, 1959. It is considered that this report concludes the work on this Contract. If this is not the case, we shall appreciate your advising us of any action that should be taken to complete the work of this Contract.

Very truly yours,

H. A. Stewart

H. A. Stewart
Assistant Director of Research

HAS:ks

Enc. - 6

cc: Chancellor John T. Caldwell- Enc. 1 (Final Report)
Mr. J. G. Vann, Business Manager- Enc. 1 (Final Report)
Dr. S. B. Tove - Enc. 10 (Final Reports: 1 signed, 9 unsigned)
(Mrs.) Jane Miller - Enc. 1 (Final Report)

UNITED STATES
ATOMIC ENERGY COMMISSION

IN REPLY REFER TO:

ORS:JDB

Oak Ridge, Tennessee
October 25, 1960

Dr. Samuel B. Tove
Department of Animal Nutrition
North Carolina State College
Raleigh, North Carolina

Subject: CONTRACT NO. AT-(40-1)-1324

Dear Dr. Tove:

Reference is made to H. A. Stewart's letter of September 13, 1960, submitting the final report under Contract No. AT-(40-1)-1324. The report has been determined to be satisfactory and concludes the obligations of the College except for a certification as to inventions made under the contract. You will receive an Inventions Certificate from our Patent Branch for completion in the near future. We have processed the voucher for payment in the amount of $950 for the remaining ten percent of the agreed consideration under Modification No. 6 and will proceed to close out the contract.

Your cooperation is appreciated.

Very truly yours,

for C. S. Shoup
Chief, Biology Branch
Research and Development Division

CC: J. G. Vann, North Carolina

UNITED STATES
ATOMIC ENERGY COMMISSION

IN REPLY REFER TO:
MCP:DSZ

Oak Ridge, Tennessee
November 15, 1960

Dr. Samuel B. Tove
Department of Animal Nutrition
North Carolina State College
Raleigh, North Carolina

Subject: CONTRACT NO. AT-(40-1)-1324

Dear Dr. Tove:

Enclosed, in triplicate, is a Certificate as to Reports, Notebooks and Inventions for the above contract. Dr. Shoup's letter of October 25, 1960 mentioned that the Certificate would be sent to you for completion.

If you believe that you have made any inventions under your contract with the A.E.C., please indicate to this office briefly the nature of such inventions and we will furnish you the necessary forms for reporting the invention in more detail. Our records indicate that no inventions have been reported thus far.

We find that the reports listed on the Certificate have been received by the A.E.C. Please list on the Certificate any papers presented or reports prepared in addition to those which we have listed.

Please execute the Certificates before a Notary Public and return two signed copies in the enclosed postage-free envelope. The third copy may be retained for your files.

Very truly yours,

D. S. Zachry, Acting Chief
Patent Branch
Oak Ridge Operations

Enclosures:
1. Certificate (3)
2. Envelope

CERTIFICATE AS TO REPORTS, NOTEBOOKS AND INVENTIONS

BY

NORTH CAROLINA STATE COLLEGE
(Contractor)

Prime Contract Number AT-(40-1)-1324

State of _____)
) ss.:
County of _____)

Samuel B. Tove (Contractor's Representative) being duly sworn, deposes and says:

1. That as the Research Professor (Title of Contractor's Representative)

of North Carolina State College (Contractor) he is fully acquainted with the work performed by the employees of the contractor under said contract.

2. That in the course of the work performed under the contract identified above the following technical reports have been submitted to Dr. Herman M. Roth (Individual Recipient), Oak Ridge Operations Office, U. S. Atomic Energy Commission or to

_____ (Individual Recipient), _____ (Organization) at _____ (Location).

Such reports are identified as follows:

TITLE	NUMBER
Progress Report, April 1952	
Progress Report, April 15, 1953	
Progress Report, March 29, 1954	
Progress Report, March 1955	
Progress Report, April 1, 1956	
Progress Report, August 1, 1957	
Progress Report, May 7, 1959	
Final Report, September 7, 1960	

3. That the following papers, covering work performed under the contract identified above, have been submitted for publication:

TITLE	JOURNAL	PUBLICATION DATE
The Turnover of Palmitic, Stearic and Unsaturated Fatty Acids in Rat Liver.	Journal of Biological Chemistry: 218:275	1956

4. That all notebook records and other original technical data recorded in the course of the work under the contract identified above are maintained in the custody shown below, and will be submitted to the Assistant General Counsel for Patents, U. S. Atomic Energy Commission, Germantown, Maryland, upon request by him for use in the preparation of patent documents or for use as evidence in establishing priority of invention. All such notebooks and other original records are listed and identified, and their present custody is shown below:

RECORD IDENTIFICATION	CUSTODY
Turnover Curves	S. B. Tove

5. That to the best of my knowledge and belief there were no inventions or discoveries made or conceived in the course of, in connection with, or under the terms of the contract identified above other than those which have, prior to the date of this affidavit, been reported to the Patent Branch of the U. S. Atomic Energy Commission, as identified below:

AEC CASE NO.	INVENTOR	TITLE
None		

6. That there were no subcontracts under this contract.

None

_____
(Contractor's Representative)

Subscribed and sworn before me this _____ day of _____, 19_____.

_____
Notary Public

(S E A L)

INSTRUCTIONS

1. In the event that there were no reports such as indicated in Section 2, above, write the word "NONE" following Section 2.
2. In the event that there were no papers such as indicated in Section 3, above, write the word "NONE" following Section 3.
3. In the event that there were no notebooks or other original data such as indicated in Section 4, above, write the word "NONE" following Section 4.
4. In the event that there were no inventions or discoveries reported as indicated in Section 5, write the word "NONE" following Section 5.