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MONSANTO CHEMICAL COMPANY
CLINTON LABORATORIES
P. O. BOX W
OAK RIDGE, TENNESSEE
721403

February 4, 1948

To: Dr. Shields Warren, Interim Director, Division of Biology and Medicine, Atomic Energy Commission, Washington, D. C.

From: Alexander Hollaender, Biology Division, Clinton National Laboratory, Oak Ridge, Tennessee.

Attached is the program of the Biology Division for 1948-49. The program for each group is written up by the individual group leader after discussion with me. I am submitting these in the present form because I feel that each group leader should have an opportunity to present his own case. Although the major goal of this laboratory is the understanding of the mechanism of radiation effects, including the intelligent use of radioisotopes, we feel that each individual investigator should have considerable freedom as to how he approaches the problem. It is the responsibility of the director to coordinate the entire program. It will be noticed that some of the group leaders have planned considerably ahead, others visualizing only problems to be attacked in the immediate future.

I should like to point out that the prevailing upset conditions at Clinton National Laboratory are reflected in these plans. In spite of the fact that the Biology Division has considerable independence in the set-up of this Laboratory, the Division will nevertheless reflect the status of the program of the entire Clinton National Laboratory. Should it be possible to have strong physics and chemistry divisions here, the attraction and holding of outstanding biologists would be easier. We sincerely hope that it will be possible to put Clinton Laboratories on an even keel in the near future. In any case we shall not weaken in our endeavor to make this a strong Biology Division.

Projects in the Process of Being Set Up or Planned For.

There are two projects which have not received complete approval as yet. 1) The Mouse Genetics Experiment, and 2) The Biorganic Project. We have had conversations with Dr. H. J. Muller and Dr. Sewall Wright in regard to the Mouse Genetics Experiment. We also had an opportunity to discuss this program with Dr. Donald Charles of the Rochester Project. We strongly feel that it would be worth while to go ahead on this project. We respectfully request that approval be given for this project as soon as possible. The other project, still under discussion, is the program in Biorganic Chemistry. This program is being discussed in great detail in the attached outline. It is absolutely essential for us to have a number of organic chemists in our division if our isotope work is to progress. The unsettled conditions of Clinton Laboratories have made it difficult to proceed as originally outlined. We are now submitting a very much modified program and feel that we should like to proceed more slowly in the building up of the project. We would like to suggest that this project be approved in its present modest form and the actual arrangements be left to the Director of Clinton Laboratories.

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Training Program

Since the Committee met here, we have endeavored to formulate a more definite training program in connection with our laboratory and have planned along the following lines:

1. Weekly Seminars. Up to the present time these seminars, which meet on Thursday afternoons at 3:30, have been given by our division members who speak on their respective fields. Beginning with the second week in February, ten to twelve seminars on basic physical phenomena in connection with the effects of radiation on living matter will be given by members of the physics and chemistry divisions. A list of these seminars is attached.

2. Radiochemistry Class. Beginning with the first week in March, a class in radiochemistry under the direction of Dr. Paul C. Tompkins will be started; four members of our staff taking part in the instruction. The class will be given twice a week and will last about three months. Should this course be a success, we should like to repeat it with outside investigators, probably under the auspices of the Oak Ridge Institute. I will keep you informed of the progress of this work.

3. Discussion Groups. We are starting a number of small discussion groups on highly specialized fields in radiation biology, which will meet twice a month. The first group will emphasize cytogenetics work. Another group is planned for the biochemists.

4. Training for Army Medical Men. A number of officers from the Army Medical Corps are now in training here. We should like to extend this training group and make it more formalized.

5. Thesis Research. We are now completing arrangments in cooperation with the Oak Ridge Institute for a program of training for men who have completed their class work to continue work on their theses in our laboratory.

6. Oak Ridge Institute Radiochemistry Program. We are cooperating with the Oak Ridge Institute in their training program in radiochemistry next summer.

7. Southeastern Universities Program. We are attempting to help the Southeastern Universities in setting up stronger biology departments. Our work at the University of Tennessee has been quite successful. We hope to make similar approaches with Vanderbilt and Emory Universities.

Weak Aspects in our Project.

1. Medical Men. We have very few medical men in our group and are urgently in need of adding two or three with interest in basic research. It is difficult to attract such men because of the modest medical facilities at

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Oak Ridge. Should it be possible to attract men on leave of absence from medical schools, we would earnestly solicit your cooperation and the cooperation of the medical men on the Advisory Board in attaining this end.

2. Physicists. There are now only a few physicists on our staff. The weakening of the physics and chemistry departments through the reorganization of Clinton National Laboratory makes this an urgent problem. We hope very much that it will be possible to hold a number of the outstanding physicists and chemists of Clinton National Laboratory. It will be most important that we have close cooperation with these men and that the physicists and chemists who remain at Clinton National Laboratory get more thoroughly acquainted with the problems in the Biology Division. We have made arrangements to bring this about.

3. Biorganic Chemistry Group. We are submitting a request for permission to set up a biorganic chemistry group. In the event that this request is not approved, we should like to add a number of organic chemists to this Division. In any case, this has now become a very difficult, although urgent, task and I am not yet certain as to our manner of proceeding in regard to it.

Size of Division.

We have at present 26 PhD.'s or M. D.'s, 16 Masters, and 15 Bachelors in our Division. We would like during the coming year to increase this group of professional people to about 80. (The attached table includes people coming here on thesis and fellowship work). However, we are not certain whether this will be necessary. In any case, we would like approval so that if the opportunity comes, we would not be handicapped in proceeding the development of the work of our Division.

Budget.

We spent during the last year $154,000 on salaries; $160,000 for equipping this laboratory; and $90,000 for permanent type of equipment; our present budget calls for $400,000 in salaries for next year; $150,000 for temporary equipment, and $100,000 for permanent equipment. It might be possible for us to get along on a budget of about $600,000 to $700,000.

Building Changes.

Changes in our second floor of the four-story building are now in progress. We have requested certain changes on the third floor of the four-story building so that we could use this area for high level C^{14} work. This is closely tied up with the organic chemistry group.

We have not received approval as yet for altering the first floor of Building 9210 for the Mouse Genetics Group. This alteration would cost around $100,000, and would make space available for this group at Y-12. If it should be decided to proceed on this experiment on a much smaller scale and then later to continue on the full-planned program, much money would be wasted in making changes for two programs instead of one.

cc: C. N. Rucker
J. C. Franklin, AEC.

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SEMINARS

February 12 - S. DeBenedetti - "Interaction between Radiant Energy and Matter"

19 - S. DeBenedetti - "Interaction between Radiant Energy and Matter"

26 - S. DeBenedetti - "Cosmic Radiation"

March 4 - A. M. Weinberg - "The Chain Reactor as an Experimental Tool"

11 - A. M. Weinberg - "Elements of Neutron Physics"

18 - A. O. Allen - "Effects of Ionizing Radiation on Liquids"

25 - A. O. Allen - "Effects of Ionizing Radiation on Liquids"

April 1 - Sidney Siegel - "Effects of Ionizing Radiation on Solids"

8 - A. S. Householder - "Biophysics of Cell Division"

15 - C. G. Shull - "Neutron Diffraction"

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PERSONNEL

	Present Status					Proposed Status				
	Ph.D	M.A. M.S.	B.A. B.S.	Assts.	Total	Ph.D.	M.A. M.S.	B.A. B.S.	Assts.	Total
Cytogenetics										
Giles	3	2	1			3	2	3		
Kimball	1	1	1			2	1	3		
Carlson	1	1	1			2	2	2		
Hollaender	1			2		2	1		3	
Stapleton		1		1	17		1		2	29
Microbiology										
Arnold	2	1				2	1			
Carson	3	1	1			4	2	3		
Anderson	1	1	1			1	2	2		
Stone	1	1			13	1	2			20
Physiology & Pharmacology										
Kohn	2 M.D.		3	1	6	3 or M.D.	1	3	2	9
Biochemistry										
Carter	4		2			4	2	4		
Tompkins	2	2	2			4	3	4		
Sheppard	1		1		14	1	1	1		24
Biorganic Chemistry	2	2	2		6	4	3	3		10
Mouse Genetics										
Russell	2	3		5	10	3	6		11	20
TOTAL	26	16	15	10	66	36**	30*	28*	18	112

* Includes men to come in for training under the Oak Ridge Institute.

** New members will be, to a considerable percentage, people transferring from other divisions of Clinton Laboratories.

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CYTOGENETICS - N. H. Giles, Jr., (Group Leader), A. D. Conger, H. J. Teas
Dorothy L. Giles, Ann E. Flynn, Josephine S. Gower.

A. The proposed research program in cytogenetics for this group may be divided into two complementary, though distinct subdivisions. The first subdivision deals with cytological studies of induced chromosomal rearrangements in Tradescantia. The second is concerned principally with genetic and biochemical studies of induced mutations in Neurospora.

I. Cytological Studies of Radiation-Induced Chromosomal Rearrangements in Tradescantia. This program is to be carried on in cooperation with Dr. Alan Conger.

1. Comparative studies of the effects of fast and slow neutrons obtained from uranium fission. The investigation has two principal aims: (a) to make possible comparisons with previous studies on the effects of fast neutrons obtained in cyclotron experiments; (b) to help elucidate the relative biological importance of the various nuclear capture reactions taking place in cells irradiated with slow neutrons.

2. Studies of the effects of various radioisotopes, especially those which can be absorbed by the plant, in inducing rearrangements. These experiments are to be carried on in cooperation with Dr. Bolomey.

3. Investigations of the effects of various physical and chemical treatments combined with radiations in modifying the frequency of chromosomal rearrangements. Combinational treatments contemplated at the present time include supersonic vibrations, anaerobiosis, infra-red, and nitrogen-mustard.

4. An attempt to obtain uranium fission within cells and to determine the range and ionization efficiency of the resulting fragment pairs in producing rearrangements.

II. Genetic and Biochemical Studies of Radiation-Induced Mutations in Neurospora. This program is to be carried on in cooperation with Dr. Howard Teas.

1. Studies of certain selected genetic loci (concerned with biosynthetic mechanisms) for mutation from normal to mutant and reverse. If suitable techniques can be developed, as seems probable, it should be possible to obtain data on the kinetics of mutation for specific genetic loci, utilizing a variety of radiations.

2. An analysis of spontaneous and induced resistance to several antimetabolites, including drugs, antibiotics, and other substances of medical importance.

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3. The accumulation, in connection with the above experiments or by separate radiation treatments, of biochemical mutants for a study of animo-acid biosynthesis. For such studies, the use of radioactive or heavy isotopes is contemplated.

Personnel

	PhD.	Masters	Bachelors
Present Status	3	2	1
Proposed Status	3	2	3

1156164

CYTOGENETICS, Cont. - R. F. Kimball, (Group Leader), Nenita Gaither, Mary K. King, Margaret E. Griess.

B. This group plans to investigate four different aspects of the effects of radiations upon the ciliate protozoan Paramecium aurelia. These are:

I. The production of lethal mutations in the micronuclei by ionizing radiations.

1. This investigation is already in progress. It was originally designed to critically test preliminary results with X-rays which appeared to show that lethal mutations were not produced independently in the two micronuclei which are present in this species, but rather either appeared in both or neither. Results of experiments with beta rays have not confirmed the X-ray investigation, but instead, are interpretable on the assumption that lethal mutations are produced independently in the two micronuclei. To complete this investigation, it is planned to test by appropriate breeding techniques whether the lethal changes that have been detected are in fact mutations of the micronuclei and to repeat the experiments with X-rays to make sure that there is no essential difference in the effects of the two kinds of radiation. This part of the research should be completed within about two months.

II. The inactivation of self-reproducing cytoplasmic factors by ultraviolet and ionizing radiations.

1. While the induction of nuclear mutations can be studied in Paramecium, this organism is uniquely favorable for study of the rate of cytoplasm in inheritance. For this reason, it is proposed to devote the major part of the program to investigations of the effects of radiation upon cytoplasmic inheritance. Through the work of Sonneborn and his students, the existence of a number of self-reproducing cytoplasmic factors is now more or less firmly established. Of these the most thoroughly investigated and best controlled for experimental purposes is the substance called kappa which determines the production by the animals of a substance which is lethal to other strains of the species. It is planned to investigate first of all, the inactivation of kappa by ultraviolet light of different wave lengths. It is also proposed to study the inactivation of kappa by ionizing radiations in respect to dosage, intensity, etc. Such investigations should yield valuable information concerning the nature of kappa and should provide further insight into the mechanisms of the effects of radiation upon the cell.

III. The production of mutations of cytoplasmic factors by ultraviolet and ionizing radiation.

The same general approach will be used to study mutations of kappa. Spontaneous mutations have occurred which result in a change in the kind of killing action of the kappa containing strain. The mutant forms of kappa appear to be self-perpetuating despite the fact that

no gene mutations are involved. Attempts will be made to induce mutations by ultraviolet and by ionizing radiations.

With material now available, it is possible to carry out the studies with several different kinds of kappa in several different genic backgrounds. It is proposed to do this in the belief that such a comparative study will provide a broader basic for generalization and may suggest relationships which would not appear in a more limited study.

If time permits, an extension of this type of investigation to other cytoplasmic factors will be made. However, it seems better to study one cytoplasmic factor thoroughly before attempting an extension to others.

IV. The effects of radiation upon the division of various components of the cell.

This line of investigation is to be looked upon as subsidiary but necessary for the proper planning and interpretation of experiments along the other three lines. However, for proper planning and interpretation of the work, especially that on cytoplasmic factors, it is necessary to know what effects the radiations that are being used have upon cell division. It is necessary to know whether they delay division, whether they interfere with the division of the micronucleus, and whether they affect the division of the micronucleus. Therefore, it is proposed to carry out the investigations of these matters with each of the radiations which are used. Paramecium is rather favorable material for the study of division since it is readily possible to follow individual cells throughout the division cycle. Moreover, it has an unique nuclear dimorphism so that effects upon different kinds of nuclei within a common cytoplasm are easily investigated. For these reasons, it is hoped that these studies may yield information which is not only of value in interpreting other investigations on Paramecium, but also of general interest as well.

Personnel

	PhD.	Masters	Bachelors
Present Status	1	1	1
Proposed Status	2	1	3

1156166

CYTOGENETICS, Cont. - J. Gordon Carlson, (Group Leader), Mary L. Snyder, Rachel McMaster, Mary E. Gaulden.

C. Effects of Radiations on Rate of Mitosis

Studies have been in progress for some months on the relative effectiveness of different dosage rates of gamma radiation from activated Tantalum in retarding cell division. One phase of this work has been completed, namely, the determination of the relative effectiveness of 2 r/min and 32 r/min dosage rates in doses of 8, 32, 64, 128, and 256 r. It is planned to continue these studies, using intensities of 0.25 r and as much lower as intensities can be measured, with a view to determining that intensity at which recovery keeps pace with effect. For such work we hope to continue using gamma radiation, though we may find it necessary or desirable to extend this work to gamma rays from other sources, and eventually, to other kinds of radiations. If a proper technique can be developed we intend to use alpha and beta particles for such studies.

Work is being initiated now to determine the effects of treating living cells with beta radiation by immersing them in P^{32} at different concentrations for different intervals of time. We wish to determine the doses of these radiations that produce effects comparable to those resulting from photoelectrons and Compton recoil electrons produced within the cell and to determine the relative effectiveness of different dosage rates.

The biological material that we have been using and shall continue to use is the neuroblast of the grasshopper embryo growing in artificial culture medium. In such living preparations, we can observe directly the effects on cell structure and cell division that follow the radiation treatment. It has been under consideration for some time to try to work out a culture method for cells of the cornea of the rat, so that the results obtained with the invertbrate material could be compared with those from mammalian cells.

Personnel

	PhD.	Masters	Bachelors
Present Status	1	1	1
Proposed Status	2	2	2

1156167

CYTOGENETICS - Alexander Hollaender (Group Leader), Eleanor D. Loftus, Imogene Armstrong, and S. Wollman?.

D. Effects of Radiation on Micro-organisms.

I. Considerable work has been done on the effects of P^{32} in solution on mutation production in fungi. It appeared at first as if radioactive phosphorus in solution is more effective than if the beta radiation is given from an outside source. Further investigations showed that the fungous spores concentrate the radio-phosphorus. We are now in the process of analyzing whether the radio-phosphorus in these spores can account for the relatively high mutation rate produced by the radiation from the absorbed radio-phosphorus. Similar experiments are planned with C^{14}.

These experiments require the cooperation of the radio-biochemistry department and our cytogenetic group. This work is also being coordinated with effects of radio-phosphorus on chromosome breaks in Tradescantia. (Dr. Giles)

II. We are also setting up a number of new tools in connection with different aspects of radiation work. We are in the process of setting up an ultra-violet monochromator, different types of infra-red equipment, an ultra-violet microscope, etc.

III. It is the main duty of this group to coordinate the work between the different groups, especially the cytogenetic and the radio-biochemistry groups. Since many of our people work in the highly specialized fields, it is my responsibility to bring these groups together and direct the work towards the main goal, i.e., to an understanding of the effects of radiation in living cells and possibly means of counteracting radiation effects.

Personnel

	PhD.	Masters	Assistants
Present Status	1		2
Proposed Status	2	1	3

1156168

CYTOGENETICS, Cont. - G. E.Stapleton (Group Leader), Frances Langford.

E. Effects of Radiation on Slime Molds.

I. Comparative effect of ionizing radiations on Myxomycetes - the life cycle of Physarum polycephalum is such that one can investigate the sensitivity of 4 distinct stages in its development; the sclerotial or dried stage, in which the organism is quite resistant to most physical agents; the spore stage; the myxameba stage, in which the individual gametes can be bomba rded; and the plasmodial stage, wherein the myxameba have joined together to comprise a naked multinuclear mass of protoplasm.

1. All the stages might be tested for sensitivity to the various radiations available here; beta rays, gamma rays, fast and slow neutrons.

2. The mutation rate can be investigated at the same time since there will be survivors of most exposures.

This should be investigated from the following viewpoints:

(a) Morphological - gross or microscopic structure alterations.

(b) Physiological - effects on streaming, respiration and ability to reproduce.

(c) Biochemical.

3. An interesting phenomenon, that of the necessity of light for these pigmented forms to "fruit" should be looked into, perhaps using shortwave length radiations as the source of energy.

Personnel

	PhD.	Masters	Bachelors	Assistants
Present Status		1		1
Proposed Status		1		2

1156169

MICROBIOLOGY - W. A. Arnold (Group Leader), A. S. Holt, Anna C. Newton.

F. The program for this Section is concerned with the study of effects of radiation on cell functions such as photosynthesis, respiration, fermentation and cell division.

The first experiments planned in this field are as follows:

I. Determine the wavelength dependence of U-V inhibition of photosynthesis and compare with that for cell division.

II. Determine whether the inhibition of photosynthesis is an "all or none" effect for each cell or whether there is some such small unit within the cell that is made inactive.

III. Apply U-V to parts of single cells to see if we can localize the effects on the chloroplasts or the nucleus.

IV. Study the effect of radiations on the hydrogen bacteria.

Personnel

	PhD.	Masters	Bachelors
Present Status	2	1	
Proposed Status	2	1	

1156170

G. Program for Isotopic Tracer Studies on Intermediary Metabolism. Although the present knowledge of a few catabolic systems is fairly complete (notably carbohydrate breakdown in muscle and in alcoholic fermentation), at the present time there is very little real understanding of the reactions in living matter concerned with synthesis of cellular constituents. It is our main purpose to make a study of some of these latter reactions, and especially as an adjunct to other types of investigations carried on in this Biology Division (e.g., radiation effects on genes, chromosomes, and cell division).

These latter studies will eventually resolve into specific effects of various types of radiation on certain "enzyme systems" involved in cell division and growth, and in genic control of these processes. It is planned that our studies will complement those of the Biochemistry Section (on peptides, protein synthesis, mucleic acids, and nucleoproteins), and that together these studies will very materially play a part in the eventual solution of problems in radiation biology.

I. Mechanism of Biosynthesis of Propionate from Pyruvate.

This is a 3-carbon system, and is an example of a "type reaction" wide spread among plants and animals. This reaction is thought to be an important link between carbohydrate and fat metabolism, but as yet no intermediates have been found and no tenable mechanism has been postulated. We are using microbial enzyme systems to carry out this reaction, since here the reaction goes at reasonably high concentration.

II. Biosynthesis of Acetate (in the propionic acid fermentation).

In the few cases now known concerning the formation of acetate from pyruvate, acetyl phosphate has been found to be the intermediate. So far, all attempts to demonstrate acetyl phosphate in the present reaction with the propionic acid bacteria have been unsuccessful (Lipmann, personal communication - not published).

Because of the importance that acetyl phosphate has assumed as one of the key intermediates in synthetic reactions, it is highly desirable to demonstrate whether there is another, as yet unknown, reaction leading to the formation of acetate. It is still possible that the proper conditions have not yet been attained to demonstrate acetyl phosphate in this fermentation. In either case it is important to study this reaction, as it probably leads directly into Section III.

III. Biosynthesis of Succinic Acid.

There are at least two possibilities for this synthesis: (1) the coupling of CO_2 with a three-carbon intermediate, with subsequent reduction, and (2) the coupling of two two-carbon pieces (acetate, acetyl phosphate?). One or both of these mechanisms may function, and the two-carbon labile intermediate discussed in Section II is probably closely connected.

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The formation of succinate also occurs in animal tissues (liver, brain, kidney, etc.). The resulting four-and six-carbon systems have assumed large importance as catalytic systems in the pathways of both catabolism and anabolism, and thus leads as a logical introduction to Section IV.

IV. Cellular Synthesis.

By making use of specific microorganisms which synthesize appreciable quantities of certain storage materials, it has already been indicated that one can learn a great deal concerning the mechanisms of these syntheses. It is proposed to make a study of several types of assimilation, both fermentative and oxidative.

Personnel

	PhD.	Masters	Bachelors
Present Status	3	1	1
Proposed Status	4	2	3

1156172

MICROBIOLOGY, Cont. - E. H. Anderson (Group Leader), M. L. Morse, Ruth Weileman.

H. Radiation Studies on Bacteria and Bacteriophage.

It is planned to carry out radiation studies on two types of material, bacteria and bacteriophage. The bacterium to be used for a major portion of the work is Escherichia coli, Strain B. A great deal of data has been accumulated for this particular strain by numerous investigators and it is the sensitive host for a well defined group of bacteriophages. The bacteriophages to be used in the phage studies are the group of seven members for which the B strain of E. coli is host. Other species of bacteria will be used where their characteristics show them to have advantages.

I. A study of the action of radiations on bacteria.

1. A comparison of the efficiencies of different radiations in killing bacteria under conditions as nearly possible similar. To the present, beta rays have been used exclusively. Other types, gamma rays, neutrons, and x-rays, will be investigated as soon as facilities are available and techniques are developed. It is planned to determine survival curves and sensitive volumes for the different radiations and compare them in different species of bacteria.

II. A study of bacterial mutations induced by radiation.

1. Variation in cellular and colony characteristics - these criteria, such as colony pigmentation, would offer a rapid means of detecting mutations and measuring mutation rates in a comparison of the efficiency of different radiations in causing bacterial mutations. Such organisms as Serratia Marcescens are to be tested.

2. Biochemical mutations with respect to nutritional deficiencies.

3. Mutations with respect to bacteriophage resistance.

The use of bacteriophage as a screening or selecting tool offers the opportunity of quantitatively analyzing an enormous population with great efficiency since all resistant cells can be isolated after rapid lysis of the sensitive or non-mutant cells.

4. Mutation rates.

Comparison of mutation rates induced by different types of radiation.

5. Investigation of a possible correlation between mutations to bacteriophage resistance and mutations to growth factor deficiencies.

6. Metabolism studies on bacterial mutants.

1. Growth rates.

6. Cont.

2. Synthetic capacity of the mutants in addition to specific growth factor deficiencies, especially a study of nitrogen metabolism of mutants resistant to phage and deficient for growth factors.

7. A study of the effects of assimilated radio-active phosphorous.

III. A study of the action or radiations on bacteriophages.

Although mutation rates of plant viruses have been shown to be increased by X-irradiation, a study of the action of radiations on bacteriophages has largely been restricted to an analysis of the inactivation of phage particles by different types of radiations and to a determination of sensitive volumes. On the other hand studies on the mutation of bacteriophages have been confined almost entirely to normally occurring spontaneous mutations. The latter studies have indicated that bacteriophage mutations occur mainly or exclusively during the period in which the particles are within suitable host cells.

Because it is expected that bacteriophages may be similar in organization to genes it is important to study the genetic behavior of these "elementary" particles and to determine whether or not their rate of mutation may be increased by radiations. (The criteria to be used in detecting bacteriophage mutations are alterations in: (1) host range, (2) plaque type, (3) lysis inhibition, (4) antigenic specificity, (5) latent period, and (6) burst size.) Such studies should yield a great deal of information on the nature of mutation.

It is planned to undertake, as far as is possible, the following program:

1. Determination of inactivation curves for the various types of radiation for the group of seven phages for Escherichia coli, strain B.

2. A study of the possibility of inducing mutations in extracellular phages.

3. A study of the possibility of inducing mutations in intracellular phages.

4. A study of the possibility of inducing mutations in phages in the presence of bacterial host cell products.

5. A study of the possibility of inducing mutations in phages by infecting host cells that have developed in media containing suitable radioactive metabolites, i.e., P^{32}

Personnel

	PhD.	Masters	Bachelors
Present Status	1	1	1
Proposed Status	1	2	2

1156174

STABLE ISOTOPES - W. G. Stone (Group Leader), T. A. Love.

I. Tracer technique has become a very powerful tool in chemistry and biology. For much of this work, radioactive isotopes are available and extremely convenient. For some purposes, such as experiments with people, and for some few elements, stable isotopes must be used.

The biologically important elements, nitrogen and oxygen do not have radioactive isotopes with convenient half lives, but do have naturally occurring stable isotopes which can be separated with comparative ease.

Conventional mass spectographs may be used for the following stable isotopes. Only the best are adequate for determining N_{15} and O_{18} percentages, particularly in biological work. N^+_{15} and CH^+_3 differ in mass by only .89%. CH_3 is a common decomposition product of diffusion pump oils and many biological products. O^+_{18} and $H_2O^+_{16}$ differ in mass by only .063%. Water is very hard to get rid of in any practical equipment and in any biological material. Mass spectographs capable of readily and accurately determining percentages of N_{15} and O_{18} have several serious drawbacks.

For these reasons, it is believed to be very important to develop a cheaper, simpler and more convenient method and apparatus for quantitative determination of isotopic content. The approach which seems most promising at present, utilizes the resonance of an ion in a uniform magnetic field. This involves frequencies near or below broadcast frequencies where conventional radio equipment and techniques can be applied. It requires magnetic volumes so small and electrode configurations so simple that with mass production of the "tube" it will be cheap enough to be considered expendable with each determination.

Personnel

	PhD.	Masters	Bachelors
Present Status	1	1	
Proposed Status	1	2	

1156175

PHYSIOLOGY AND PHARMACOLOGY - H. I. Kohn (Group Leader), Mary B. Cupp, J. J. Lane**, Capt. P. W. Robinett, Germaine Click, Sara Giles, Nancy J. Swingley.

J. Radiation Sickness.

I. This section is oriented toward work of clinical interest and therefore has the point of view of experimental therapeutics and medicine. The fields of endeavor fall under the following headings, but it is to be emphasized that these are not mutually exclusive:

1. Experimental Irradiation Sickness.

These studies are directed at the pathologic physiology of mammals subjected to whole body irradiation.

(a) Check on the reported therapeutic effect of parabiosis, i.e., that the shielded member of such a pair of rats can protect the exposed member.

(b) Studies will begin shortly on the effects of low dosage upon the immunologic and endocrine systems, and the interaction between these.

2. Neoplastic Disease.

Skin tumors are readily produced by beta rays. We shall continue the study of this convenient preparation by examining

(a) The dose-exposure area relationship.

(b) The effect of physical factors (temperature, circulation, etc.).

II. Experimental Therapeutics - We are interested in all methods of treatment; the effect of rutin is now being examined, (on Dr. Warren's suggestion). It is also planned to study the effect of irradiation upon inflammatory processes in conjunction with the studies listed under 1.

During the course of the projected work we expect to employ the usual methods of hematology, histopathology, pharmacology, physiological chemistry, serology, etc.

Personnel

	M.D.	PhD.	Masters	Bachelors	Assistants
Present Status	2			3	1
Proposed Status	3 or	3	1	3	2

** On leave of Absence.

1156176

BIOCHEMISTRY - C. E. Carter (Group Leader), W. E. Cohn, Capt. N. S. Cooper, Marian W. Kies, Ann R. Webster.

K. Biochemistry of Nucleo-Proteins and Enzymes.

This group will undertake three general classes of problems. A study of the metabolism of nucleic acids in normal and irradiated tissues and *in vitro* experiments upon isolated nucleic acids designed to indicate possible mechanisms of radiation injury to genetic material in terms of altered physico-chemical properties of nucleoprotein and nucleic acids.

The metabolism of nucleic acids will be studied by experiments correlating quantitative determination of tissue nucleic acid, enzymatic activity of systems responsible for the degradation of nucleic acids (nucleases, nucleotidases, etc.), and the rate of turnover of the elemental constituents of nucleic acids as indicated by isotopic analysis.

I. Investigation of Biochemistry of Genetic Mechanisms.

1. If radiation injury to nuclear structures is principally implemented through altered nucleic acid metabolism, then it should be possible to delineate the enzymatic systems involved, and by suitable analytical techniques to describe the biochemical results of the injury. Experiments employing isotopic radio-phosphorous will be conducted in conjunction with Dr. Cohn.

2. Desoxyribonucleic acid and nucleoprotein present striking physical characteristics: high anomalous viscosity and assymetry, and high molecular weight. Several experiments to date upon irradiated nucleic acid suggest that alteration in these physical properties induced by radiation may be responsible for the genetic effects of radiation injury. It is important to determine whether these effects which have been described *in vitro* can be established for material isolated irradiated nuclei, and to what extent the experiments upon isolated nucleic acids reflect changes in fundamental structure of the native molecule, or of its aggregated state in colloidal aqueous solution.

To carry out such experiments a study will be made of the following properties as related to radiation exposure:

(a) Electrophoretic properties will be studied to determine whether radiation changes the net charge on the molecule and in particular, whether the ability to combine with protein as indicated by homogeneous migration in an electric field is altered.

(b) sedimentation and diffusion behavior to establish size, shape, and molecular weight.

(c) viscosity studies to estimate the size and assymetry of the molecule.

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(d) titration studies to assess possibilities of dissociation through internucleotide phosphate linkages and through hydrogen bonding at the animo groups.

It is proposed to conduct parallel experiments utilizing ultrasonic vibration to degrade the nucleic acid by non-specific methods and thus serve as some control on disaggregation effects. The physical chemical studies will be carried out in conjunction with Dr. Volkin.

II. Because of the striking sensitivity of bone marrow and testis to radiation injury, the altered metabolic states induced by such injury will be studied. Transamination reactions are probably key mechanisms of protein metabolism and involve a coenzyme, pyridoxine, which exhibits a marked sensitivity to some forms of radiation. For these reasons transamination will be the first system studied, but other systems, particularly those involved in assimilatory functions will be investigated. These tissues will also be included in the nucleic acid studies.

III. This group intends to continue earlier studies in enzyme systems involved in intracellular protein metabolism, i.e., dehydropeptides and peptides with an amide linkage adjacent to the peptide bond. These systems exhibit extraordinary activity and suggest that the classes of compounds involved are of fundamental importance in protein metabolism.

IV. In conjunction with Dr. Bolomey we have undertaken experiments designed to establish the degree of toxicity of beryllium to the mammalian organism and through isotopic techniques follow its pathway of assimilation and detect the organic compounds into which it is formed.

Personnel

	PhD.	Masters	Bachelors
Present Status	4		2
Proposed Status	4	2	4

RADIO-BIOCHEMISTRY - P. C. Tompkins, (Group Leader), R. A. Bolomey, J. X. Khym, Jo E. Gardner, Leon Wish, Venus I. Knobf.

L. Proposed Program for the Radio-biochemistry Group covers the following:

I. Training Program in Radiochemistry and Radiochemical Techniques.

1. Lecture course in elementary radiochemistry, counting, properties of radiation, and principles of hot techniques.

2. Practical training in radiochemical laboratory techniques at both tracer and semi-hot levels.

3. Training film (collaboration with Health Physics) covering same ground as Section II.

II. Applied Radiochemistry and Radiochemical Analysis.

1. Urinalysis for important radio-isotopes (e.g. Sr^{89}, Ba^{140}, P^{32}, I^{131}) - (with Health Physics).

2. Mechanism of action of P^{32} solution in mold mutations. Calculation of dose and determination of P^{32} distribution (with A. Hollaender).

3. Analysis of P^{32} in anthers of plant buds and calculation of dosage as related to chromosome breaks. (with N. Giles)

III. Biochemistry of Mineral Metabolism.

1. Analysis of Be in blood and urine.

2. Relation of chemical species of Be to the metabolism of Be, Be toxicity. Involves determination of chemical formulae, and dissociation constants of Be complexes as they occur in body tissues.

IV. Development of new micro-methods for the separation, purification and estimation of nucleic acids and their components.

V. Fundamental radiochemistry necessary for the development of problems which will fall under section II and III.

1. Development of standards for absolute counting rate determinations, gamma-ray energies, beta-ray spectra, and decay schemes of any isotopes whose radiations may be of interest as either internal or external radiation sources.

2. Study of the properties of radiations, and the mechanisms of their absorption by matter in general and the light elements of biological interest in particular.

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3. Standardization and monitoring all sources needed for exposure purposes (including the pile flux at site exposure).

	Personnel		
	PhD.	Masters	Bachelors
Present Status	2	2	2
Proposed Status	4	3	4

BIOCHEMISTRY - Radiation of Erythrocytes - C. W. Sheppard (Group Leader)
W. R. Martin.

M. An Investigation of the Effect of Ionizing Radiation on the Cellular Physiology of Erythrocytes.

I. The mammalian erythrocyte is an extremely simple cellular system and since it does not possess a discrete nucleus, is of particular fundamental biological interest. The effect of ionizing radiation on such cells is of secondary clinical importance only. However, from the standpoint of fundamental biology, such effects are important indeed. It has been found by previous workers that high doses of radiation (over 10,000 r.) produce serious disturbances in their osmotic equilibrium with surrounding plasma. This has been attributed to alteration by the radiation of the membrane permeability. We now propose to investigate this phenomenon more completely.

II. Previous extensive studies of the permeability of the normal erythrocyte membrane to electrolytes have been hampered by inadequate experimental techniques. The availability of radioactive isotopes now permits studies of the transfer of such electrolytes as sodium or potassium between the cell and its surroundings without disturbing the normal equilibrium in any way. The project will commence with an investigation of the exchange of sodium and potassium *in vitro* between red cells and plasma. The isotope of K^{42} of 12.4 hour half-life is available in sufficiently high specific activities. By the use of this isotope and simultaneous potassium analyses, it will be possible to investigate permeability to potassium quantitatively. Later permeability to sodium will also be studied. The radiation experiments will include the use of an improved radiation source, consisting of a cylindrical piece of gold foil, suitably supported, which can be activated in the pile. Although present studies will be devoted to dog erythrocytes, other species may be investigated subsequently.

III. It is anticipated that the work of this project will include more than a study of radiobiological effects alone. Several factors involved in the maintenance of normal osmotic equilibrium should eventually be examined in order that radiation effects can be properly understood.

The proposed work will be conducted in close cooperation with similar investigations elsewhere and in particular with studies now in progress at the Institute of Radiobiology at Chicago University. Close attention will be paid to research now being done on membrane permeability at other institutions. A visit to several laboratories is now being planned with this end in view. Discussions with Dr. M. Jacobs at the University of Pennsylvania, Dr. A. K. Parpart at Princeton, Dr. Eric Ponder, Nassau Hospital, and Drs. T. P. Ting, and Raymond E. Zirkle at the University of Chicago are being arranged.

BIOCHEMISTRY, Cont.

	Personnel		
	PhD.	Masters	Bachelors
Present Status	1		1
Proposed Status	1	1	1

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N. Isotopic Organic and Biorganic Chemistry Project

I. General Scope and Objectives

A rational solution of basic problems in radiation biology leads directly into the problems involved in radiation sickness. In this Biology Division the groups studying cytogenetical effects of radiation in microorganisms, plants and animals will depend only too soon upon basic research carried out by others of our groups in the fields of enzyme and nucleic acid chemistry and intermediary metabolism. The success of these latter projects in turn depends heavily upon development of practical methods of both synthesis and degradation of isotopically labelled organic compounds.

It is felt that such an organic chemistry program must be carried out and integrated within this Biology Division for at least two reasons, (1) in order that the groups mentioned above may make immediate and practical use of the contributions from the organic group, and (2) in order that the program of research in the organic group can be developed along lines which are eventually most useful to Biology.

Therefore, in order for this Biology Division to utilize isotopes to their fullest advantage, it is essential to initiate research along the following lines: First, to develop methods for synthesizing organic compounds containing labelled atoms in specific locations; and secondly, to develop analytical procedures (i.e., unequivocal degradation processes and satisfactory radiochemical assay methods).

Furthermore, widespread dissemination of these useful methods and special techniques to universities and industry depends upon a training program within this organic chemistry group.

II. Specific Integration of Objectives

An integral part of the biology program is concerned with the biochemistry of cells and tissues. Biological tracer studies require organic compounds containing labelled atoms in known positions. These molecules are converted by biological systems to products whose chemical and isotopic structures (i.e., the position of the labelled atoms within these structures), must be determined. In order to determine unequivocally the positions of the labelled atoms in both the starting materials and the products, these compounds must be degraded to fragments by known procedures; these depend upon a knowledge of the reaction mechanisms involved.

Without this knowledge it is not only impossible to synthesize the labelled compounds, but it is equally impossible for the biochemists and biologists to interpret their experiments. Eventually, therefore, a synthetic-organic program pays off in two directions at the same time: it furnishes methods by which necessary labelled compounds can be prepared, and it furnishes the biochemists and biologists with the necessary knowledge of reaction mechanisms, in order that they can use these compounds to their fullest advantage. Hence the present plan is to have a well integrated

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organic and biorganic program with the necessary personnel to solve these inter-dependent problems, and to therefore be in a most advantageous position to cooperate and consult with the biochemists.

III. Necessary Techniques

Certain techniques and methods peculiar to C^{14} organic synthesis have been worked out in the Chemistry Division of the Clinton National Laboratory and are available.

1. Excellent methods for the combustion of organic compounds to carbon dioxide.

2. Methods to determine radioactivities by ion current measurements.

3. Procedures for synthesis of a number of organic compounds as well as some key intermediates (which are necessary as starting materials).

IV. Personnel

It is planned to start with two or three senior organic chemists, preferably with experience in C^{14} organic synthesis. With approval of this program, there is an excellent chance to obtain the services of several such people.

Within the first year, it would be planned to gradually build the group up to five or six organic chemists.

V. General Requirements for Space and Facilities

1. Needed space

(a) Laboratory space of at least 1000 sq. feet, equipped with chemical benches, services, and hoods. An adjacent room which could be used for instruments.

(b) In regard to high level work, not only is additional space required in order to set up the necessary vacuum racks, but very adequate ventilation facilities are needed (both from a health viewpoint as well as that of maintaining a sufficiently low background of radioactivity for technical reasons.

2. Present space and facilities in Bldg. 9207 (Y-12) which could be made available for this program:

The "needed space" and many of the necessary facilities could be made available for both low and high level work.

(a) In the area available for low level work are two chemical laboratories with the following facilities:

(1) 180 ft. of stainless steel (top) chemical benches

(2) 42 ft. of hood space

Both of above with necessary services

(3) 750 sq. ft. of free space useful for placing movable frames carrying ventilated vacuum racks (which are indispensable for C^{14} synthetic work); service lines are available overhead.

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(4) Several small rooms off these main laboratories. One is equipped with hoods, and one is equipped as a constant temperature instrument room.
(5) Also adjacent is a storage room containing floor-to-ceiling enclosed cabinets.

(b) Completely separated by a wide hallway from the main laboratories is an area of 2500 sq. feet which could be used for the high level synthesis. (There is also room in this area to set up an adequate protected high pressure hydrogenation set-up). This area of the building is already equipped for an enormous rate of air change, and ventilating ducts are available for hood facilities at eight locations in this area.

(c) Office space for technical personnel could also be made available.

Personnel

	PhD.	Masters	Bachelors
Present Status	2	2	2
Proposed Status	4	3	3

MOUSE GENETICS - W. L. Russell (Group Leader), Liane B. Russell, J. C. Kile, Rowland E. Logan, W. J. Muller (Univ. of Ind.)*.

C. Mammalian Genetics Program.

I. Determination of mutation rate at certain chosen loci - this method is being used on Drosophila by Dr. Muller at the present time. It gives accurate data on the mutation rate at a limited number of loci. The number is limited only by the size of the experiment and by the number of known genes which can be tested practically. One strain of mice already homozygous for six known recessive genes could be used as soon as facilities are available.

II. Determination of mutation rate of genes with detectable effects on a wide variety of anatomical and physiological characters - the objective in this project is to obtain as many as possible of all types of mutations with detectable phnotypic effects. To "capture" the recessives efficiently, it will be desirable to carry the experiment to the fifth generation at least and then follow certain lines through several later generations. By extensive diagnostic and autopsy examinations, refined as far as possible, mutations effecting a wide variety of anatomical and physiological characters would be detected.

III. Determination of cumulative effects of minor mutations - the breeding plan in this project is to maintain several lines of descent from a single inbred strain. Some of these would be kept as controls, while the rest would be irradiated in each generation. After several generations, all lines would be studied and compared, as populations, for differences in a large number of quantitative characters, e.g., growth rate, disease resistance, tumor incidence.

IV. Determination of somatic mutation rate - if valid comparative mutation rates for somatic and germinal tissues can be established, then the determination of somatic mutation rate, which, for certain genes, can probably be made more easily, could be used as a faster method for evaluating the genetic dangers of radiation.

Importance to human genetics - although tolerance levels, so far as physiological effects are concerned, are being rapidly established, very little is known about the effects of radiation on mutation rate in mammals. All of the above projects are, therefore, important separately. Only as a group, however, will they make possible a reasonably complete evaluation of the genetic hazards to man. Such an evaluation is not possible at the present time. Information on some organisms, e.g., Drosophila, is excellent in some respects, but is entirely lacking, or very limited, in others, e.g., in the field of investigation proposed in the third project. Furthermore, the morphology and physiology of mammals are quite different from that of organisms whose mutation rates have been studied intensively. Even if we knew accurately how the rate of mutation in mammals compared with that in Drosophila, we could not, without the type of experiments outlined here, estimate the effect of a given induced mutation rate on the morphological and physiological health of mammalian populations in successive generations.

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MOUSE GENETICS, Cont.

It is estimated that the program as outlined will require an average of 3,000 mouse cages during the first year, 6,000 for the second and 10,000 for the third.

Personnel

	PhD.	Masters	Bachelors	Assistants
Present Status	2	3		5
Proposed Status	3	6		11

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