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EFFECT OF RADIATIONS ON MICROORGANISMS AND CERTAIN BIOLOGICAL SYSTEMS

Progress Report 1

By

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EFFECTS OF RADIATIONS ON MICROORGANISMS

AND CERTAIN BIOLOGICAL SYSTEMS

INTRODUCTION

The known electromagnetic radiations can be arranged on a wavelength or frequency scale into an electromagnetic spectrum. This spectrum is divided into several regions which are not fixed as to their points but are arbitrary. In general, the divisions are based on the methods that are used to produce the radiations; it is possible to produce a given frequency by two methods. An example of this may be noted with ultraviolet light, which is produced by a gaseous discharge, having a wavelength of 10^{-6} centimeters the same wavelength that can be obtained by x-rays.

Alpha, beta, and gamma radiations of radioactive substances, of x-rays, and of protons and neutrons are "ionizing" radiations. Ultraviolet light, on the other hand, is nonionizing radiation, but it causes "excitation" of the materials exposed to this form of energy. Practically all the energy that is dissipated by radiations in tissues ultimately becomes degraded to heat energy. Thus, a dose of 100,000 roentgens (r) is sufficient to raise the temperature of a target about 0.25°C. The effect of this increase in temperature appears insignificant. Yet when the object is considered as a whole, instead of as individual atoms of a particle, the summation of temperature increase may have a definite biological effect in sensitive microscopic systems.

Ionization as well as excitation radiations produce chemical changes in objects treated with electrons. X-rays and ultraviolet light are electromagnetic radiations emitted in "quanta", but differ in wavelengths (0.05 to 10Å (angstroms) for x-rays, and 2,000 to 3,000Å for UV) resulting in their being little similarity in practice.

Gamma rays are produced by x-rays, radium, or certain fission products of uranium. The absorption in tissues by gamma rags is practically all by electron recoil. X-rays and gamma rays produce their effects in tissues by the projection at high speed of electrons already present in the tissues. However, electrons may be introduced into tissues of thin sections from an

external source such as beta rays, which are fast-moving electrons emitted from radioactive materials, from cathode rays, which are artificially accelerated electrons. Beta rays can also be produced from radon gas. Finally, there are alpha rays, which are the nuclei of helium atoms emitted spontaneously by radioactive materials and can also be produced by the cyclotron.

Ultraviolet Light

About 0.1 per cent of the sun's energy reaching the earth is in the form of ultraviolet light. A low-pressure mercury-vapor sterilizing lamp can efficiently convert approximately 17 per cent of its total electrical energy into mercury resonance radiation of 2537Å, which is very effective in the destruction of bacteria, molds, protozoa, viruses, and other forms of life. Ultraviolet light does not penetrate crevices, opaque liquids, or ordinary glass; thus its application for sterilization of materials is limited.

Ultraviolet rays between 2540 and 2800Å in length are the most effective with respect to germicidal activity. Below 2400Å the coefficient of absorption is increased but the rays are shorter and less germicidal. Different bacteria have been found to respond to rather specific wavelengths of light. Ehrismann and Noethling[1] noted that ultraviolet of 2650Å is most lethal for Micrococcus candidus, Saccharomyces cerevisiae, Pseudomonas aeruginosa, Staphylococcus aureus, and Vibrio finkler, whereas Serratia marcesens was most sensitive to 2805Å. Duggar and Hollaender[2] noted, moreover, that tobacco mosiac virus was 100 times more sensitive to light of 3100Å than to 2652Å.

Gram-negative, nonspore forming bacteria are more susceptible to ultraviolet light than are the gram-positive organisms. Next in order of resistance are bacterial spores, while viruses appearing extremely resistant to the radiation.

Temperature has little, if any, effect on ultraviolet activity. Organisms that have been exposed to the rays may show an increased sensitivity to heat and also ordinary light. Cells which are apparently unaffected by the rays appear to develop normally, while those exposed to lethal doses of ultraviolet often lose their refractivity and degenerate into shadowy forms. Sublethal effects may cause the cells to become markedly enlarged, some appearing as filament forms and others showing prolongation in time of cell division.

The use of ultraviolet light radiation in the preparation of bacterial vaccines was early discouraged, probably due to unreliable light sources and other uncontrollable methods of exposure. The conventional method of using phenol for the preparation of rabies vaccine results in a material being poor in immunizing properties. Thus, Hodes, Webster, and Lavin,[3] using controlled ultraviolet light irradiation of rabies suspensions

for 45 minutes, found that the viruses became avirulent for mice and yet retained 10 times as much immunizing properties as chloroform treated virus, and 500 times as much activity as phenolized vaccine. Sarber, Nungester, and Stimpert[4] found that a virulent human strain of tubercle bacillus could be rendered nonviable by ultraviolet irradiation and yet was as antigenic as BCG (Bacillus of Calmette-Guerin) as evidenced by guinea pig protection tests. Of particular importance, the stability of the irradiated vaccines was at least a matter of months as compared with days for BCG vaccine.

A more recent application of ultraviolet light is in the irradiation of blood products for destruction of the causative agents of infectious hepatitis and homologous serum jaundice. Both these conditions are transmitted by the parenteral administration of blood, blood products, and vaccines.[5,6,7] Several factors, such as poor penetrability and instability of the blood, have greatly limited an extensive use of this method for the destruction of the agents responsible for the infections.[8]

Mutation and chromosome changes have been noted in microorganisms that have been exposed to ultraviolet.[9,10,11] Evidence is available to show that by the irradiation of relatively poor antibiotic-producing strains of penicillin and streptomyces cultures, the latter can be changed to mutants which produce larger and more potent amounts of antibiotics.[12,13] Another interesting observation in ultraviolet studies is that organisms that have been exposed to sublethal doses of light can be "photoreactivated" by subsequent exposure to ordinary light.[14,15] This phenomenon has materially changed the general concept of the lethal effect of ultraviolet radiations on microorganisms.

Radium and Radioactive Isotopes

The effects of radium and alpha and beta radioactive isotopes upon microorganisms appears to be inhibitory rather than germicidal. *Serratia marcesens* has been exposed to 20 mg of radium with only an inhibitory action on the multiplication of the organism.[16] The same has been found to be true when exposing *Eberthella typhosa* and *Vibrio cholera* to radium bromide at a distance of 1 cm from the latter.[17]

Radioactive phosphorus (P^{32}) and tobacco mosiac virus were added to the food of the tobacco plant.[18] In this instance, it was shown that nonpathogenic forms of virus could be obtained from virulent forms by the action of P^{32}. P^{32} "tagged" tobacco mosiac virus was fed to mice, whereupon the activity of the radioactive sutstance was found to be localized in the liver and spleen.[19]

A study was made of the effects of radioactive phosphorus and iodine (I^{131}), as well as x-ray, on a variety of fungi.[20] This study was prompted by observations that certain samples of radioactive isotopes

received in the laboratory for experimental use were found to be contaminated with fungus spores. Of 42 strains of varieties of fungi studied, only 11 of the number were destroyed <u>in vitro</u> by x-rays (1100r), and none by treatment with P^{32} and I^{131} (20 microcuries).

In the same study some of the irradiated pathogenic and nonpathogenic fungi were inoculated intravenously into animals to check the organisms for any possible alteration in pathogenicity. <u>Alternaria</u>, a nonpathogen could be followed by the Geiger counter after injection, and when the animals were sacrificed after 15 days no lessions were found, nor could the organisms be recovered from any of the tissues. The animals receiving the pathogenic organism, <u>Blastomyces dermatitidis</u>, died in 24 days or less, of generalized blastomycosis. The distribution of this organism could also be followed in the animal body by the Geiger counter. Evidence was also presented to show that the radioactive materials were actually absorbed on the spores and mycelia of the fungi.

The major portion of work that has been carried on during the past few years on radioactive isotopes in experimental biology has been on the use of these agents as "tracers". In immunological studies, I^{131} has been used to "tag" the globulin fraction of antisera and then studied in serological reactions.[21] By this means it has been found that tyrosis forms an essential part of the antigenic determinants of horse serum globulins. Evidence is also available to show that there is localization of radioactivity in the kidneys of rats and mice following the administration of a radioactive-iodine-labeled globulin fraction of an antiserum prepared against homologous kidney tissue.

Brief mention has been made in the literature of the use of radioactive tracers in the determination of the mode of action of germicides and chemotherapeutic agents upon bacteria. Previous studies have well established that the oxidation of metabolites by living cells involves the passage of hydrogen or electrons, or both, along a series of oxidation-reduction systems. Oxidation in this chain of reactions occurs only at the terminal steps involved in the cytochrome-oxidase system. Succinic acid dehydrogenase has been used along with deuterium to label the carbon-bound hydrogen atoms of succinic acid, and methylene blue used as the hydrogen acceptor for this succinic oxidation <u>in vitro</u>.[22] In the direct transfer of atoms in this reaction it has been found desirable to label the hydrogen atoms of succinic acid, and then after incubation with an acceptor (methylene blue) to isolate the acceptor and determine the deuterium content.

Strains of yeasts which were unable to oxidize acetate were found to metabolize isotopic acetate ($CH_3C^{13}OOH$) and produce a large increase in fat.[23] The marked increase in fat, demonstrated using the heavy carbon, was the direct result of assimilation of the intact acetate molecule. The acetate appeared to have a "sparing" action on the cellular carbohydrate, since a large decrease in the stored carbohydrate occurred in the absence of the acetate.

Studies on the penicillin uptake of microorganisms by the use of radioactive penicillin (S^{35}) reveal that yeast cells do not absorb penicillin, nor does any of the antibiotic penetrate the cell wall.[24] With Staphylococcus aureus, on the other hand, two types of uptake occur: (1) a specific uptake of 0.8 u/ml that is independent of the extracellular concentration of penicillin; and (2) a diffusion of penicillin into the cell so that the intracellular water has the same concentration as the extracellular water. Neither extensive washing nor equilibration with solutions of nonradioactive penicillin released the bound radioactive penicillin from the bacterial cells.

Brief mention may be made of studies of radioactive mosquitoes followed through their migration by their radioactivity;[25] the fate of ingested calcium when fed to chickens, found to be mainly deposited in the shell of the egg;[26] the biological synthesis of radioactive silk;[27] etc. No mention will be made at this point of the use of radioactive iodine in malignancies, particularly of the thyroid gland.

High-Speed Electrons

One of the more recent applications of radiation that has received rather wide publicity is the use of high-voltage generators to discharge or accelerate electrons which have been used in the treatment of cancer (x-rays) and, of particular interest in this review, for the sterilization of foods and drugs. For this purpose, several types of machines have been constructed and all have been experimentally studied in various laboratories for their effects on living tissues, microorganisms, and various biological systems. One of these units has been described as the "capacitron" which releases penetrating, negatively charged particles (electrons) during a period of about 1/1,000,000 of a second, during which period the electron intensity applied during such short radiation impulses amounts to about 30,000 to 50,000 amperes. A description of the capacitron and its applications as a tool for sterilization and preservation of food in the raw state has been presented by Brasch and Huber.[28]

Unlike the static type of potential used in the Brasch-Huber generator, a machine, the "Van de Graff", has been developed which produces a steady and constant potential releasing 10,000 to 12,000,000 roentgen-equivalent-physicals (rep). Most of the mechanical development of electrostatic generators and studies of the effects of the electrons on tissues, microorganisms, foods, and drugs have been carried out at the Massachusetts Institute of Technology.[29-32] One of these reports describes the effects of x-rays from the Van de Graff, as well as cathode rays from the instrument, upon pure cultures of bacteria, yeasts, molds, and also upon foods, fats, vitamins, and enzymes.[32] One of the shortcomings of cathode rays is that they penetrate water or other substances to a maximum distance of only about 1 cm for each 2,000,000 volts of energy.

X-rays of 3 megavolts were found to destroy readily bacteria, yeasts, and molds in massive concentrations, in pure culture and when associated with liquid or solid substrates such as milk, water, apple juice, ground spices, soil, and catgut sutures. This order of activity is the same as that of cathode rays, but the latter are much more rapid than x-rays. Gram-positive, gram-negative bacteria and spore-forming organisms are readily destroyed by cathode rays. As one might expect, somewhat higher voltages are required to destroy the latter than are necessary for nonsporeforming or vegative microorganisms.[32]

Potatoes were blanched successfully with x-rays, although there was some destruction of oxidative and amylolytic enzymes. The peroxide oxygen values of butter and olive oil are slightly increased by this radiation. There is a gradual decrease in the reduced form of ascorbic acid with increasing doses of x-rays. Similar trends, but at a slower rate were shown when orange and grapefruit juices were x-irradiated. However, it was indicated that most of the ascorbic acid was oxidized only to the dehydroascorbic acid stage. More concentrated solutions of the vitamin were found to be more sensitive than solutions containing such added materials as glucose, proteins, etc. Goldblith, Proctor, Hogness, and Langham irradiated niacin tagged with C^{14} in the carboxy group with 3,000,000-volt cathode rays and reported that retention is a function of dosage.[33] The retention varied from 94 per cent for 66,000 rep to 45 per cent for 2,640,000 rep. X-rays appear to have little effect on niacin with dosages up to 1,000,000 roentgens.[34]

In sterilization procedures with electron radiation, the general rule is that higher organisms are easier to destroy than lower ones (bacteria vs viruses). A weevil (ounce for ounce) can stand about 100 times as much electron radiations as a man. Bacteria are ten times more durable than weevils. Thus, while it looks practical today to use electrons in flour mills to kill weevils, a full bactericidal dose may damage complex organic molecules in foods. While many simple substances like penicillin or surgical sutures are unharmed by an electron barrage, food products which contain complex molecules usually suffer damage.

In the work of Brasch and Huber[28] which was published a year prior to the MIT paper, the workers subjected the following items to high-frequency impulses of their instrument ("capacitron"): bacterial suspensions, diphtheria antitoxin, whole blood, blood plasma, protein hydrolysate, penicillin, novocaine solution, brewer's yeast, bran, proteolytic and diastatic enzymes, as well as foodstuffs such as meat, sea foods, dairy products, vegetables and fruits. Insofar as the effects on the blood constituents, enzymes, and drugs were concerned, they reported that sterility was affected on these products with no loss in potency or damage to the chemical compounds. With respect to foods, however, Brasch and Huber noted that while many of the products could be sterilized by treatment with high-velocity electrons, in general this was accompanied by a change in color, texture, taste and, in some instances, odor of the foods which proved to be objectionable to many individuals.

In a subsequent publication these authors found that while profound changes would occur in bovine plasma albumin in the liquid state when exposed to 7,000,000 rep in the form of high-speed electrons, the same dose of irradiation at -50°C has little effect on the albumin.[35] They also pointed out that cooling of foodstuffs before treatment with electrons would exclude the undesirable side reactions of taste, color, and texture. Treatment of materials while under vacuum will also give the desired effects without side reactions.

Brasch and Huber concluded their report by indicating that B. mesentericus spores could be destroyed by 11,000 rep, whereas the same effect was noted with 120,000 rep of the conventional source of beta, gamma, or x-rays. With the virus of mouse encephalomyelitis, the inactivation dose of electrons produced by the capacitron was 35,000 rep, and for tobacco necrosis virus 2,800,000 rep. With vaccinia virus, the dose required for inactivation with short electron impulses of high intensity was 11,000 rep, whereas 100,000 rep were required with beta and gamma rays to produce the same effect. The dosages required to kill viruses are much higher than those required to kill bacteria, which may be explained by the "direct hit" theory because the viruses are of much smaller size. Although these are about the same size as the viruses, bacteriophages, which are parasites of bacteria, require slightly lower dosages for sterilization. The phages investigated by Brasch and his coworkers required doses of 600,000 and 650,000 rep for 100 per cent sterilization.

Huber[36] found that enzymes were entirely unaffected in foods that were fully sterilized by radiation. However, these foods remained in their natural state and showed no noticeable change in taste, odor, or appearance after long periods of storage. Thus, it appears that while the enzymes show a positive test for activity, they do not perform their usual role in promoting food spoilage in the irradiated samples. Huber offers two possible explanations for this unusual effect; first, inactivation may have occurred at a point of the enzymatic chain which is not reflected in the testing procedure; or second, the extent as well as the speed of the enzymatic breakdown of the food without the assistance of microorganisms has been considerably overestimated.

Nickerson, Goldblith, and Proctor[37] treated fish tissues with 3,000,000-volt cathode rays and found that sterilization may be attained but that protolysis, the enzymatic protein degradation, was not affected.

Proctor and Goldblith[34] found that when mixtures of niacin and ascorbic acid were used the vitamins exhibited mutual protection. Anderson and Harrison,[38] using relatively concentrated solutions of ascorbic acid (0.5 mg per 100 ml), reported a reduction of the vitamin concentration by a factor of one-half by treatment with x-rays doses of 5,000 roentgens. Dunlap and Robbins[39] noted no definite effect of 200 kilovolts of x-rays on thiamine chloride. The dosages in the latter instances were quite low, being of the order of 10,000 roentgens. With beta radiations from radioactive phosphorus and radon, however, a definite inactivation was obtained.

Traub, Friedemann, Brasch, and Huber[40] described the preparation of rabies vaccine in which the virus was inactivated with high-intensity electrons from the capacitron. The vaccine thus prepared was bacteriologically sterile; it was found to be superior to phenolized vaccines and compared well insofar as its antigenicity was concerned with vaccines prepared by ultraviolet inactivation.

X-rays

The use of x-rays in experimental biology has been studied very extensively since their discovery by Roentgen in 1895. The rays of wavelengths greater 1Å are classified as "soft" rays, which are readily absorbed by materials. Those having wavelengths less than 1Å are known as "hard" rays, due to their greater energy they are able to penetrate farther into substances than soft rays. Bacteria and viruses can be killed by x-rays, although their resistance to the effects of the latter is quite great. It is known that when metals are in close proximity to the bacterial cells, exposure of the organisms to x-rays becomes more lethal. Trillat[41] attributed the latter effect to the protoelectrons emitted from the metals. Pauli and Sulger[42] noted that the bactericidal activity of x-rays is 80 per cent higher at 41°C than 37°C. The addition of fluorescent dyes to the suspending medium has also been found to increase the lethal action of the radiation against microorganisms. Furthermore, exposure of culture media to ultraviolet rays will render the environment unfavorable for the growth of certain bacteria.[43]

Schneider[44] found that when actively fermenting cultures of yeast were exposed to x-rays the organisms lost some of their ability to ferment carbohydrates. Rice and Guilford[45] were able to demonstrate that radiation of rapidly growing bovine _Mycobacterium tuberculosis_ would result in a change of colony form from "rough" to "smooth", and that large doses of x-rays proved lethal for the organisms. Insofar as the nonacid fast organisms are concerned, these bacteria changes in colony morphology were not induced by radiation. Haberman and Ellsworth[46] noted that when a culture is exposed to x-rays death proceeds at a logarithmic rate and the lethal action is dependent more on the wavelength emitted than on the intensity, the short wavelengths (hard rays) being more effective in killing _Staphylococcus aureus_ than are the long wavelengths (soft rays) of the same intensity.

Brace[47] exposed yeast cells to 20,000 r of x-rays and found that the cells and their descendents attained a mean volume of more than two times that of those not radiated. However, there was no corresponding difference in their specific gravities nor in their nitrogen contents.

Fram, Proctor, and Dunn[48] treated _Staphylococcus aureus_, _Escherichia coli_, _Aerobacter aerogenes_, _Serratia marcesens_, _Pseudomonas aeruginosa_, and _Pseudomonas fluorescens_ with 50,000 electron-volts of x-rays and found that the first organism was the most resistant to the radiation. They also demonstrated that the percentage of bacteria killed by a specific total dose was

the same regardless of the initial concentration of bacteria in the suspension. Their results indicated that the bacteria were destroyed by soft x-rays according to the "direct-hit" theory of radiation.

Lea[49] defines the lethal dosage of radiations as that amount which will kill 64 per cent of the original number of bacteria. He found that for vegetative organisms the dosage required increased in the following order; beta rays, gamma rays, hard x-rays, soft x-rays, neutrons, and alpha rays. With bacterial spores the alpha rays are most lethal, probably because the latter produce high specific ionization. Some workers have suggested that the spores are a dual complement of genes, both of which must be inactivated in order to inhibit multiplication. This may explain why 100 per cent sterilization of spores takes about twice the dose required for 100 per cent sterilization of vegetative cells.

Dunn Campbell, Fram, and Hutchin [32] radiated molds with high-voltage x-rays and found that a 100 per cent kill was obtained with Aspergillus niger by 250,000 to 500,000 roentgens, while a dosage of only 50,000 r destroyed 99 per cent of the same organism. However, when they radiated a species of the genus Mucor, a dosage of 1,000,000 r killed all the molds and a dose of 500,000 r destroyed 99 per cent of them. Thus considerable variation in sensitivity to radiation can be expected among the different mold species as with bacteria. Yeasts appeared to be only slightly more resistant to x-radiation than the nonsporeforming bacteria.

Dale[50,51] found that certain enzyme systems could be decreased in activity by exposure to x-rays. Using radiation doses of the order of tens of thousands, he noted that some enzymes (carboxypeptidase) resisted inactivation in the presence of their substrates (materials whose reactions the enzymes catalyze.) He also found a significant protective effect on the enzymes by the presence of carbohydrates and fats, and that dilute solutions of enzymes are more easily inactivated than are concentrated solutions.[52,53,54] Forssberg confirmed the findings of Dale and pointed out the environmental conditions are to be regarded as a critical factor in enzyme inactivation, slight changes reportedly yield dosage differences for equivalent inactivation of as much as 100 per cent.

Tytell and Kersten[55] studied the effects of x-ray wavelength, initial enzyme activity, and radiation temperature on enzyme inactivation. Longwave x-rays were found to be most effective and more absorption of the rays occurred at low temperatures (6-8°C). They noted that the per cent of inactivation was a function of the initial enzyme activity; the higher the initial activity, the higher the per cent of inactivation. In general most investigators concerned with the effect of radiation on enzymes agree that inactivation is an exponential function of dosage. Forssberg, however, although corroborating the expression for "high" concentrations of enzymes, offers a linear relationship for "low" concentrations. Yet he fails to offer a clear definition of high and low concentrations of enzymes.

Pollard[56] described his enzyme inactivation experiments using deuteron bombardment against dry pepsin, trypsin, and chymogen. He gave an exponential relationship for survival with dosages reported in terms of deuterons per sq cm. Penicillin was little affected by the radiation.

One of the theories that has been advanced for the mechanism of enzyme inactivation in aqueous media is the formation of activated water molecules with subsequent reactions with the enzyme. This would suggest that the enzyme is inactivated by a secondary effect of the radiation. An extensive review of the radiochemistry of aqueous solutions has been prepared by Weiss.[57] Zimmer[58] disagrees with the activated-water hypothesis and believes that the "direct-hit" theory applies to the inactivation of enzymes as it does in the case of bacteria.

Barron,[59] working with the sulphydryl enzymes, considered that the enzyme itself is not destroyed by radiation but that the active groups in the same enzyme molecule are modified. Barron, James[60], and Hevesy[61] suggest that some of the changes that accompany the radiation of enzymes may be caused by changes in the colloidal properties of these compounds, since in "solution" most enzymes are dispersed colloidally.

In general, the inactivation of vitamins and other drugs appears to parallel closely the activation of enzymes. The same order of magnitude of dosages for any significant inactivation, and identical protective effects, dilution effects, etc., have been observed for both classes of compounds. It would appear, therefore, that both enzymes and vitamins would remain relatively unaffected by radiation dosages required for 100 per cent sterilization. Thus, dosages appreciable higher than 1,000,000 roentgen are probably necessary to attain any significant degree of inactivation of enzymes.

EXPERIMENTAL

A. THE EFFECTS OF RADIATIONS UPON BACTERIAL METABOLISM

1. Introduction

The initial research program instituted under the general Michigan Memorial-Phoenix Project 20 involved a consideration of the biological effects of ultraviolet radiations on a well-defined and essential medium for the propagation of a specific strain of microorganism. As the work progressed and source of gamma radiation in the form of cobalt-60 (1,000 curies) was procured by Engineering Research Institute Project M943, under the supervision of Professor L. E. Brownell, the program was extended to a consideration of the effects of gamma radiations on various other biological systems. The latter aspects of this research are the subject of the second and major part of this progress report. (Page 22)

2. The Effects of Ultraviolet Radiation on a Bacteriological Medium

In order to study what effect radiation of a medium has on bacterial growth, a group of experiments was performed in which a medium of known composition was radiated. The medium used was that employed for the microbiological assay of niacin, using *Lactobacillus arabinosus* 17-5 as the test organism. The medium contains all the necessary growth factors except niacin (nicotinic acid), which when added in appropriate concentrations gives a linear growth response with the organism which then can be measured turbidimeterically. Since all components of the medium are essential for growth, alteration of one or more will interfere with the proper growth of the organism. The composition of the medium is as follows:

Difco Niacin Assay Medium (Niacin-Deficient)

Component	Amount
Bacto-vitamin free casamino acids	12 gms
Bacto-dextrose	40
Sodium acetate	20.0
l-Cystine (Difco)	0.4
dl-Tryptophane	0.2
Adenine sulfate	0.02
Guanine	0.02
Uracil	0.02
Thiamine hydrochloride	0.002
Calcium pantothenate	0.002
Pyridoxine hydrochloride	0.004
Riboflavin	0.004
p-Aminobenzoic acid	0.002
Biotin	0.0000008
Dipotassium phosphate	1.0
Monopotassium phosphate	1.0
Magnesium sulphate	0.4
Sodium chloride	0.02
Ferrous sulphate	0.02
Manganese sulphate	0.02

For the experiments, the dehydrated medium was dissolved in appropriate amounts of distilled water and 5 ml dispensed into tubes and autoclaved at 15 lbs (121°C) for 15 minutes.

A stock reference standard of niacin was made up by dissolving 50 mgm of niacin in 500 ml of absolute alcohol. This stock solution was stored at 5°C. A reference standard for each experiment was made up aseptically by diluting 0.5 ml of the stock reference standard with 500 ml of sterile distilled water. In the preparation of a standard curve showing the dependence of *L. arabinosus* on niacin for proper growth, varying concentrations of the vitamin were added to 5 ml of the test medium as follows:

0.5 ml	equivalent to	0.05 gamma	of niacin
1.0 "	"	0.10 "	" "
1.5 "	"	0.15 "	" "
2.0 "	"	0.20 "	" "
2.5 "	"	0.25 "	" "
3.0 "	"	0.30 "	" "
3.5 "	"	0.35 "	" "
4.0 "	"	0.40 "	" "

Each tube then was made up to a total volume of 10 ml with sterile distilled water.

For the radiation procedure each tube contained 0.2 gamma of niacin. With this concentration good growth of the organism occurred.

The organism used in these experiments was Lactobacillus arabinosus, obtained from the American Type Collection. It was maintained by stab culture on Difco "Micro-Assay-Culture Agar" at 5°C after an initial incubation for 24 hours at 37.5°C. A new stab culture was made up each week. A suspension of the organism was prepared by inoculating 10 ml of Difco Inoculum Broth, which was then incubated at 37.5°C for no longer than 20 hours. After incubation the broth suspension was centrifuged, the supernatant liquid poured off, and the cells resuspended in 10 ml of P.S.S. One ml of this suspension was diluted 1:100 or 1:50, depending on the turbidity. One drop of the suspension was used to inoculate the tubes by means of a tuberculin syringe and a No. 19 needle.

Turbidimetric measurements were made in all cases after incubation at 37.5°C for 18-20 hours. A photoelectric colorimeter (Lumertron Model 400-G) with a No. 530 filter was used. A tube of medium not inoculated with the organism was used as a blank.

Technique of radiation was as follows: 10 ml of media containing 0.2 gamma of niacin were exposed to a Hanovian ultraviolet lamp in open Petri dishes of 9.5-cm diameter. This gave a sufficiently thin layer of medium so that there was a minimum amount of absorption. During radiation the Petri dishes were periodically rotated. In all cases the lamp was turned on for at least 10 minutes before irradiating the media in order to sterilize the adjacent area. After irradiation of the medium, it was removed asceptically and placed in sterile tubes. Since some evaporation occurred during prolonged radiation, the volumes were adjusted so that each tube contained equivalent volumes. The tubes were then inoculated and incubated. In all cases duplicate series were run.

In order to determine whether or not some of the ingredients of the medium were being altered or destroyed, the following components were made up in equivalent concentrations as they occur in the media and then added to the medium after radiation:

Niacin
1-Cystine
dl-Tryptophane
Para-amino benzoic acid
Adenine sulphate
Guanine
Uracil

The above components were selected because they represent factors which would alter the growth of the organism if they were destroyed in any way. It was theorized that if these substances were altered during radiation, the addition of them would bring the growth of the organism to the maximum level, as present in the control.

Results:

Curve I and Table I show the dependence of Lactobacillus arabinosus 14-5 on niacin for growth.

Curve II and Table II show the affect of ultraviolet light on medium on the growth of L. arabinosus 17-5. As evident from the curve, there seems to be a linear decrease of growth with time of irradiation.

Curves III-VII and Tables III-VII show the effect of adding various components after radiation. None of the ingredients added appeared to bring the growth of the organism up to that of the unirradiated control.

Discussions:

As shown by Curve II, the exposure of medium to ultraviolet light causes a change in the environment which inhibits the growth of L. arabinosus as measured turbidimetrically. Change in pH of the medium before and after irradiation of 1-1/2 hours was negligible (pH was 5.8 before, as compared to a pH of 5.8 after irradiation. Therefore, a change in pH is not responsible for the change in growth response.

Other possibilities would be the formation of some toxic products such as H_2O_2 or organic peroxides which would have a deleterious effect on the organism, or the alteration of some essential constituent which would interfere with the metabolism of L. arabinosus. The addition of niacin, dl-tryptophane, cystine, uracil, guanine, and adenine after radiation does not bring the growth up to that of the control. Hence, it could be concluded that these are not the components altered by irradiation.

TABLE I

DEPENDENCE OF L. ARABINOSUS ON NIACIN FOR GROWTH

Concentration of Niacin	Optical Density*
0.00	0.0098**
0.05	0.082
0.10	0.14
0.15	0.186
0.20	0.217
0.25	0.239
0.30	0.277
0.35	0.306
0.40	0.315

TABLE II

EFFECT OF IRRADIATING MEDIA WITH ULTRAVIOLET ON THE GROWTH OF L. ARABINOSUS

Radiation Time (Hrs)	Optical Density
0	0.222
1/2	0.189
1	0.165
2	0.111
3	0.084

* Average of duplicate tubes.

**The greater the optical density, the greater the growth.

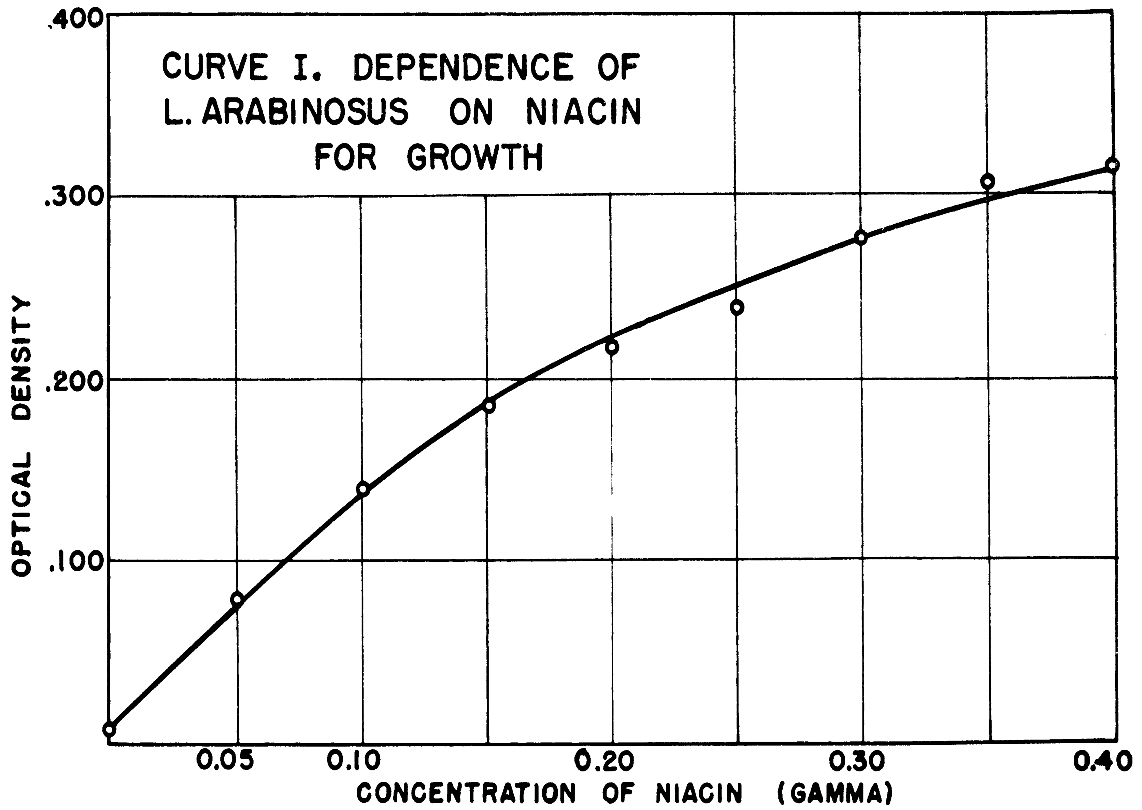

FIG. I.

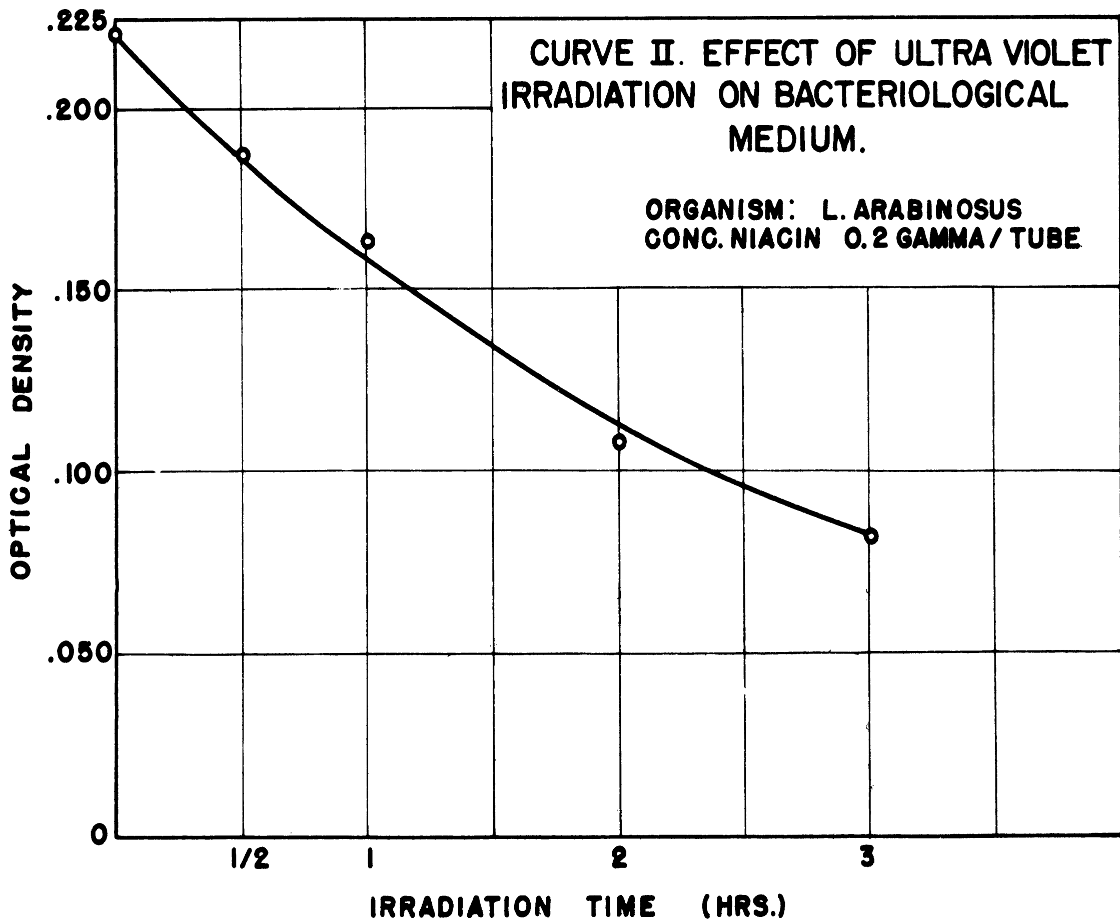

FIG. 2

TABLE III

EFFECT OF ADDITION OF NIACIN TO MEDIUM AFTER ULTRAVIOLET IRRADIATION

Irradiation Time (Hrs)	Optical Density*
0	0.256
1/2	0.220
1	0.082
2	0.097
3	0.074

TABLE IV

EFFECT OF ADDITION OF PARA-AMINO BENZOIC ACID
TO MEDIUM AFTER ULTRAVIOLET IRRADIATION

Irradiation Time (Hrs)	Optical Density
0	0.226
1/2	0.148
1	0.075
2	0.073
3	0.057

* See footnote Table II, page 14.

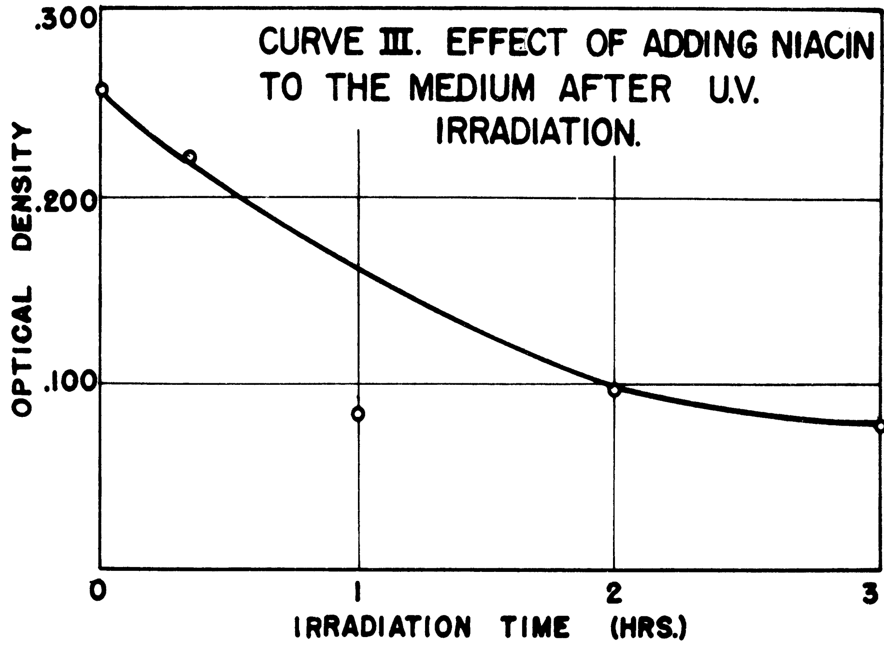

FIG. 3

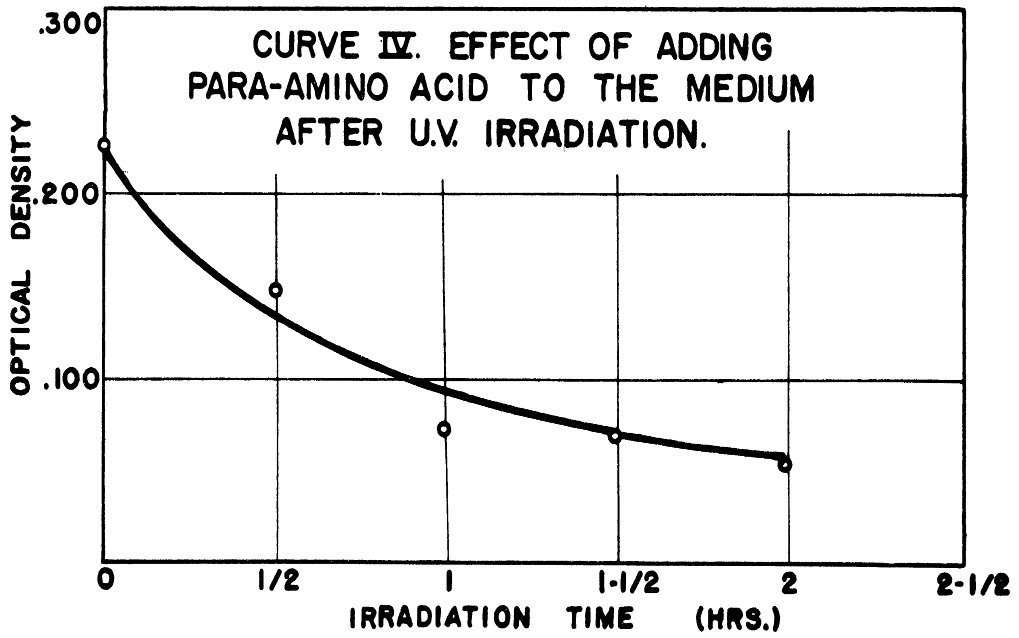

FIG. 4

TABLE V

EFFECT OF ADDING TRYPTOPHANE TO
THE MEDIUM AFTER ULTRAVIOLET RADIATION

Radiation Time (Hrs)	Optical Density*
0	0.245
1/2	0.250
1	0.201
2	0.143
3	0.106

TABLE VI

EFFECT OF ADDING CYSTINE TO THE
MEDIUM AFTER ULTRAVIOLET RADIATION

Radiation Time (Hrs)	Optical Density
0	0.256
1/2	0.215
1	0.143
1-1/2	0.143
2	0.119

* See footnote Table II, page 14.

TABLE VII

EFFECT OF ADDING ADENINE SULPHATE,
URACIL AND GUANINE TO THE MEDIUM AFTER ULTRAVIOLET RADIATION

Radiation-Time (Hrs)	Optical Density*
0	0.198
1/2	0.148
1	0.14
1-1/2	0.134
2	0.114

TABLE VIII

EFFECT OF ADDING ADENINE SULPHATE, GUANINE, AND
URACIL TO THE MEDIUM AFTER ULTRAVIOLET RADIATION

Radiation Time (Hrs)	Optical Density		
	Uracil	Adenine	Guanine
0	0.235	0.229	0.233
1/2	0.184	0.187	0.208
1	0.184	0.187	0.244
1-1/2	0.158	0.181	0.194
2	0.124	0.155	0.194

* See footnote Table II, page 14.

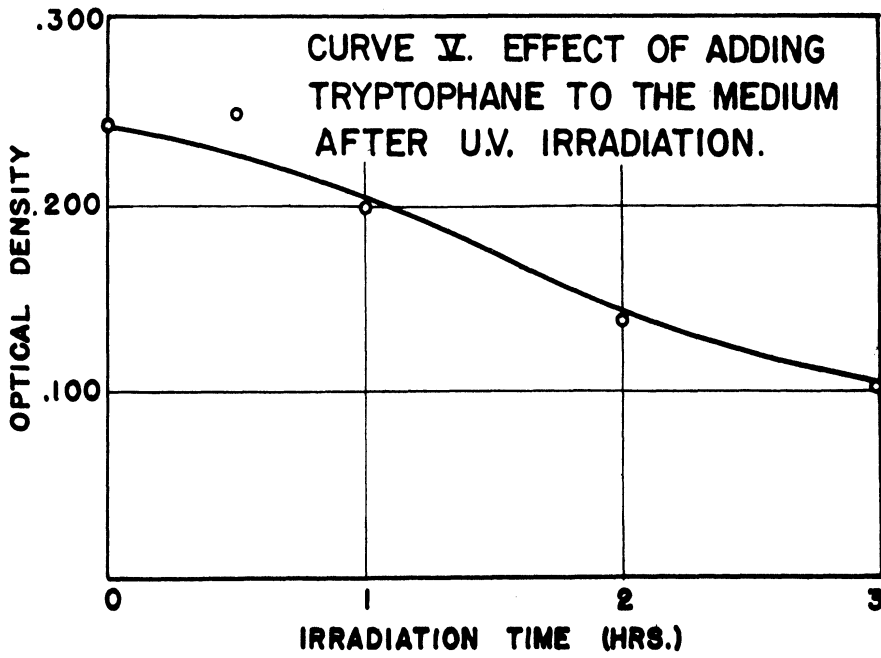

FIG. 5

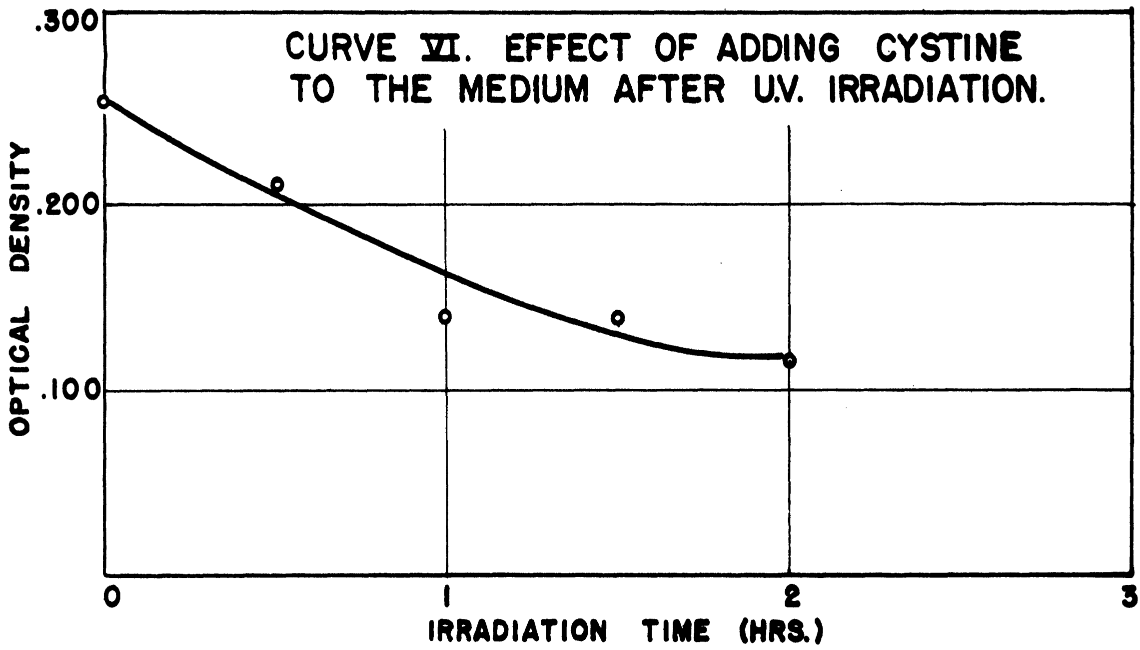

FIG. 6

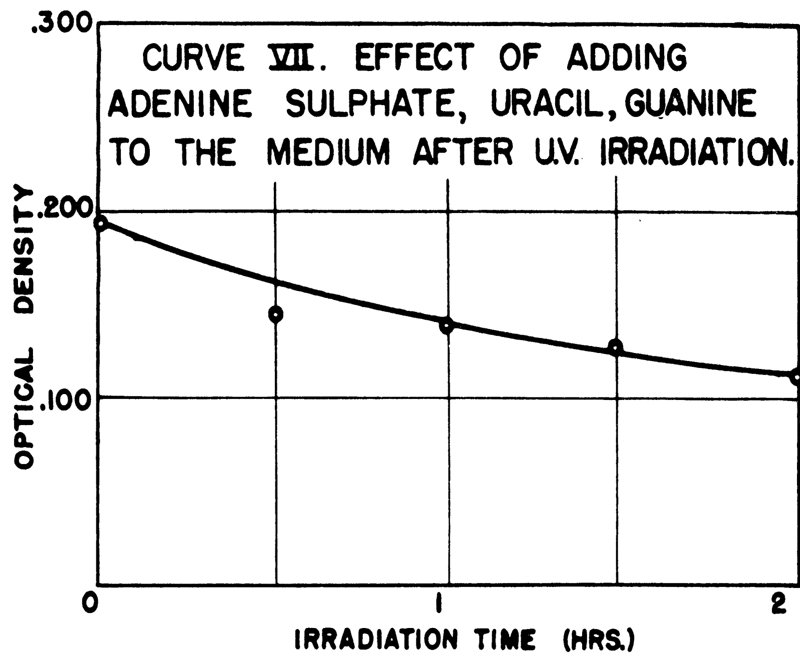

FIG. 7

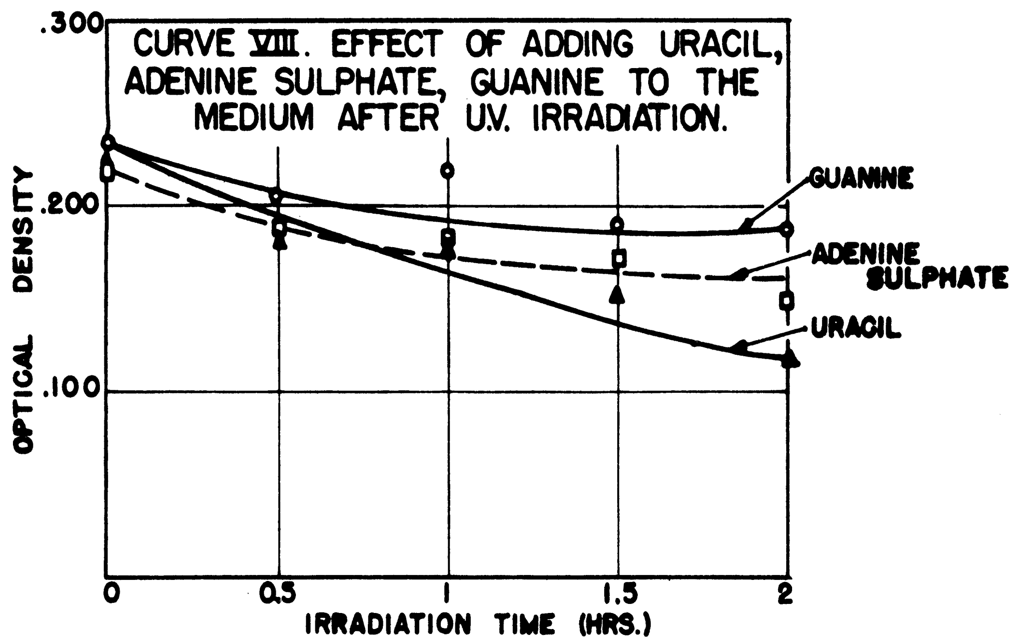

FIG. 8

B. COOPERATIVE RESEARCH WITH ENGINEERING RESEARCH INSTITUTE PROJECT M943

1. Introduction

Engineering Research Institute Project M943 and Phoenix Projects 20 and 41 have a mutual interest in the effect of gamma radiation on microorganisms, enzymes, and other biological factors. The Engineering Research Institute project is attempting to find industrial uses of the radioactive fission products and the Phoenix Project is interested in the humanitarian uses of atomic energy.

Since Phoenix Project 20 has the use of bacteriological laboratory, equipment, and personnel, and since the Engineering Research Institute has the use of a 1000-curie cobalt-60 source, it was considered advisable to pool resources on a joint research effort. Radiations are performed by Engineering Research Institute Project M943, and bacteriological tests are by Phoenix Project 20. The results of these tests are to be made available to both Engineering Research and Phoenix.

2. The Lethal Effects of Gamma Radiations (Cobalt-60) on Microorganisms

a) Experiment No. 1. The Effects of Gamma Radiation (Cobalt-60) on Proteus vulgaris, Eschericha coli and Lactobacillus arabinosus.

Cultures and Methods: E. coli and Proteus vulgaris were grown on beef extract agar slants at 37°C for 18 hours. The growths from the surface of the slants were suspended in 15 ml sterile saline solution (0.85% NaCl) containing 0.5% gelatin (pH 6.2), The suspensions were filtered through sterile glass wool and distributed in sterile pyrex culture tubes.

The culture of L. arabinosus was grown in Difco Micro-Inoculum Broth for 18 hours at 37°C, at which time the organisms were centrifuged, the supernatant medium discarded, and the packed cells resuspended in sterile saline-gelatin solution. This culture was also distributed in pyrex test tubes, as above, but since the suspension appeared homogenous the material was not filtered through glass wool.

Approximately 5 ml of each culture in 15 x 150 pyrex culture tubes (with plastic screw-caps) was exposed to gamma radiation (cobalt-60). Since previous calibrations of the temperature within the cobalt-60 vault indicated it to be comparable to the temperature of the room in which it was stored, the control culture suspensions which were not irradiated were maintained at room temperature in the laboratory during the period of irradiation of the test suspensions.

Dilution of Culture Suspensions, Plating and Counting: With but occasional exceptions, dilutions of the treated and untreated organism

suspensions with subsequent plating in agar was carried out within one hour after the cultures were removed from the cobalt-60 vault.

In the preliminary dilution series of the treated and untreated organism suspensions, 0.2-ml samples were added directly to the center of sterile Petri dishes and 0.2 ml placed in 100 ml of sterile saline solution. Then 0.2 ml of the latter dilution was added to additional sterile Petri dishes and 0.2 ml of the same suspension was put in a second bottle containing 100 ml saline. This same method of plating and dilution was carried out for an additional, or third series of tests, in which the untreated control sample was plated for bacterial counts. All cultures in which plates were prepared were made in duplicate.

To each of the 0.2-ml suspension samples in individual Petri dishes was added approximately 15 ml of melted and cooled (45°C) nutrient agar. The plates were tilted and swirled gently to mix the suspension in the medium and the agar then allowed to solidify. When the latter was solid, the plates were incubated at 37°C for 24 to 48 hours and the numbers of colonies developing in the media were counted with the aid of a Quebec Colony Counter.

The results of the first experiment against *Proteus vulgaria, E. coli,* and *L. arabinosus* are presented in Table IX.

TABLE IX

COUNTS OF P. VULGARIS, E. COLI, AND L. ARABINOSUS
AFTER 24 HOURS IRRADIATION

	P. vulgaris	E. coli	L. arabinosus
Sample (irradiated 24 hrs)	0	0	0
Control (unirradiated)	1,593,750,000	850,000	65,000

b) *Experiment No. 2. The Effects of Gamma Radiation (Cobalt-60) on Proteus vulgaris, Eschericha coli, and Lactobacillus arabinosus.*

Since the results of the preliminary experiment (No. 1) indicated that an exposure of the three test organisms to cobalt-60 for a period of 24 hours caused 100 per cent "destruction" of all the organisms, a second experiment was carried out on fresh suspensions on all three cultures, which were treated with gamma irradiation for a shorter period of time, 5 hours.

The cultures used and the method by which the test was carried out are essentially a duplication of the procedures given in Experiment No. 1. The results of the present study are given in Table X.

TABLE X

COUNTS OF P. VULGARIS E. COLI, AND L. ARABINOSUS
AFTER 5 HOURS IRRADIATION

	P. vulgaris	E. coli	L. arabinosus
Sample (Irradiated 5 hrs)	0	0	0
Control (unirradiated)	1,898,750,000	1,138,000,000	25,000

c) Experiment No. 3. The Effect of Gamma Radiation (Cobalt-60) and Roentgen Radiation (X-rays) on the Bacterial Flora of Milk.*

A quart of pasteurized whole milk was purchased on the market and distributed in polyethylene tubes. One of the samples was irradiated with cobalt-60 for 2 hours; another sample was exposed to x-rays for a period of 1 hour and 32 minutes. A control sample was placed outside and adjacent to the cobalt vault but not exposed to radiation. A second control consisted of testing the pasteurized milk for bacterial counts before the experiments were started. The methods of dilution and plating and subsequent counting are essentially the same as outlined in Experiment No. 1. The results of this study are given in Table XI.

TABLE XI

COUNT OF BACTERIAL FLORA IN MILK AFTER IRRADIATION

Sample	Numbers of Organisms per ml
M-14 (2 hrs irradiation with Co^{60})	155
M-12 (1 hr 32 min irradiation with x-rays)	165
M-11 (2 hrs standing outside of cobalt vault - control)	250,000
M-10 (Control - count on milk before irradiations)	805

* This experiment was suggested by Professor L. E. Brownell of the Engineering Research Institute under a grant of the U. S. Atomic Energy Commission, Contract No. AT(11-1)-162, Project M943. The bacteriology was carried out under the present Phoenix Project Grant, Nos. 20 and 41.

d) Experiment No. 4. The Effect of Gamma Radiation (Cobalt-60) on the Bacterial Flora of "Raw" Milk.*

While a definite reduction and inhibition of bacterial growth was obtained in Experiment No. 3, in which pasteurized milk was exposed to cobalt-60, it appeared desirable to determine whether a similar effect could be obtained by exposing "raw" milk to the radiation. In the present study the time of exposure of the milk, contained in polyethylene bags, was extended to a period of 24 hours. Appropriate controls on the unirradiated samples were maintained as indicated in Experiment No. 3. The results of this study are presented in Table XII.

TABLE XII

COUNT OF BACTERIAL FLORA IN "RAW" MILK AFTER IRRADIATION

Sample	Numbers of Organisms per ml
M-27 (24 hrs irradiation with Co^{60})	0
M-26 (24 hrs standing outside of cobalt vault - control)	4,500,000
M-28 (Control - count on milk before irradiation	20,000

e) Experiment No. 5. The Effect of Gamma Radiation (Cobalt-60) on *Proteus vulgaris* and *Escherichi coli*, with Reference to Lethal Activity in Proportion to Time of Irradiation.

Since the two preliminary or exploratory tests (Experiments 1 and 2) indicated that exposures of 5 and 24 hours of pure cultures of *P. vulgaris* and *E. coli* to cobalt-60 resulted in 100 per cent "destruction" of the organisms, it appeared desirable to determine the effects of shorter durations of exposures of the same cultures. The preparation of the culture suspenwions for irradiation, dilutions, and plate counting were carried out as outlined in Experiment No. 1. The number of surviving organisms following irradiation, the number in the unirradiated control specimens, and per cent reduction noted in the treated samples are given in Table XIII.

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* See footnote, page 24.

TABLE XIII

COUNT OF P. VULGARIS AND E. COLI AS A FUNCTION OF IRRADIATION DOSAGE

Time of Irradiation	P. vulgaris	Per Cent Reduction	E. coli	Per cent Reduction
0 (unirradiated control)	1,666,000,000	0	1,976,250,000	0
50 min	4,500,000	99.7	1,600,000	99.8
1 hr 45 min	3,680	99.99	500	99.99+
2 hr 25 min	55	99.99+	0	100.00
3 hr 40 min	0	100.00	0	100.00
5 hr	0	100.00	0	100.00

f) Experiment No. 6. The Effect of Gamma Radiation (Cobalt-60) on Sporeforming microorganisms Bacillus subtilis, A gram-Positive Sporulating Bacillus Isolated from Milk (M-S);* Penicillium notatum; and Aspergillus niger.

With the evidence obtained in the preliminary experiments (Nos. 1, 2, and 5) that cobalt-60 has a definite "lethal" action on nonsporulating bacteria (P. vulgaris, E. coli), studies were outlined to determine the effects of cobalt radiations upon microorganisms of the sporeforming varieties. Cultures used in the present investigation included a 4-day old culture of B. subtilis and a 22-hour old culture of a sporeforming bacillus originally isolated from a milk sample. Both these organisms were grown on agar slants (beef extract agar). In addition to the latter bacteria, suspensions were made of 22-day old cultures of the molds P. notatum and A. niger, which were grown on Sabouraud's Maltose agar slants. Suspensions of the organisms were made as indicated in Experiment No. 1 of this report. Microscopic examination of each speciman before irradiation revealed an abundant number of spores of the organisms. In the plating procedure in which the molds were tested, Sabouraud's Maltose agar was used. The bacteria were plated, as indicated previously, in beef extract agar. The latter organisms (colonies) were counted after 24 and again after 48 hours incubation at 37°C. The mold

* The culture of the sporeforming bacillus was isolated and obtained through the courtesy of Dr. F. W. Barber, National Dairy Research Laboratories, Inc., Oakdale, Long Island, New York.

counts were made following 5 days incubation at room temperature (24-26°C). The results of this study are presented in Table XIV.

TABLE XIV

COUNT OF SPOREFORMING MICROORGANISMS AFTER IRRADIATION

Time of Irradiation	B. subtilis	Per cent Reduction	M-S	% Red.	P. notatum	% Red.	A. niger	% Red.
0 (Control)	100,000	0	6,987,000	0	125,000	0	75,000	0
4 hrs, 25 min	19,000	81.0	7,800	99.9	0	100	10	99.98
7 hrs, 15 min	3,250	86.75	4,240	99.95	5	99.99	5	99.99

Fig. 9 shows a photograph of Petri dishes with a normal mold growth of P. notatum and A. niger on the control but no growth on samples irradiated for 4 hours.

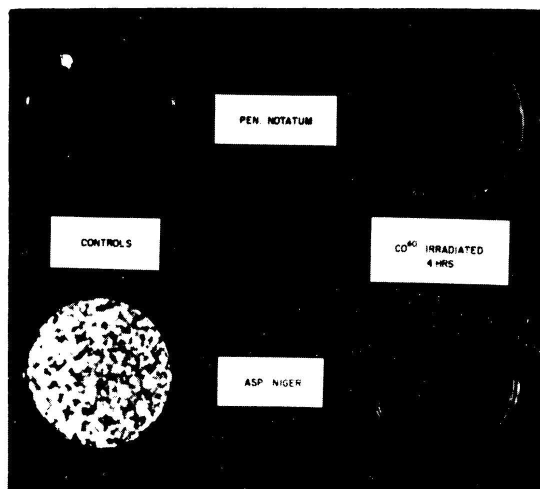

Fig. 9. Photographs Showing Effects of Gamma Irradiation on Molds.

g) Experiment No. 7. The Effect of Gamma Radiation (Cobalt-60) on Sporeforming Bacteria, Bacillus subtilis and a Gram-Positive Sporulating Bacillus Isolated from Milk (M-S)* with Reference to Lethal Activity in Proportion to Time of Irradiation.

Inasmuch as complete "destruction" of the two sporeforming bacteria B. subtilis and M-S was not obtained in the previous experiment

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* See footnote, page 26.

(No. 6) following 7 hours exposure to cobalt-60, a second study was outlined in which frequent sampling was carried out on the same organisms following irradiation with the isotope over a period of 24 hours. The suspensions used in this investigation were prepared in saline-gelatin (0.5%) solution from beef extract agar slants that had been incubated at 37°C for 96 hours. At 4-hour intervals 2-ml quantities of the specimen were removed from the irradiated samples and the latter, as well as the unirradiated controls, were stored in the refrigerator (2°C) until plated. Plating was carried out 2 hours after irradiation of the final samples (24 hours). Dilutions of the samples, plating, and counting were essentially the same as described in Experiment No. 1. Table XV presents the results of this experiment.

TABLE XV

COUNT OF SPROEFORMING BACTERIA VERSUS IRRADIATION DOSAGE

Time of Irradiation (hrs)	B. subtilis	Per cent Reduction	M-S	Per cent Reduction
0 (unirradiated)	18,000,000	0	97,250,000	0
4	960,000	94.6	8,750	99.99
8	202,000	98.8	3,280	99.99
12	20,500	98.8	550	99.99+
16	14,450	99.99	80	99.99+
20	75	99.99+	10	99.99+
24	0	100.0	0	100.0

Fig. 10 shows the difference in growth of colonies of M-S organisms after different irradiation dosages.

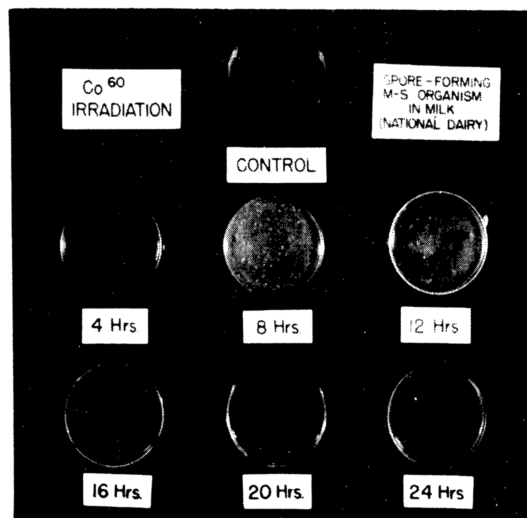

Fig. 10. Photograph Showing Effects of Gamma Radiation on Sporeforming (M-S) Microorganisms in Milk.

Since complete destruction of several cultures of bacteria was obtained in the irradiation studies up to this point, the number of survivals of the test organisms has been plotted against the dosage of cobalt-60 used in treating the suspension (irradiation time) for some of these data. Fig. 11 presents a graph of the irradiation results compiled against P. vulgaris, E. coli, B. subtilis, and the gram-positive sporulating bacillus isolated from milk (M-S), representing the results of Experiments No. 5 and 7.

h) Experiment No. 8. The Effect of Gamma Radiation (Cobalt-60) on Spores of Molds, Aspergillus niger and Penicillum notatum.

With the evidence obtained in Experiment No. 6, that the spore-forming fungi, A. niger and P. notatum, were markedly reduced in numbers following irradiation with cobalt-60, a second study was carried out on these two mold species in which the time intervals of irradiations was spaced at shorter periods than before and sampling made at hourly intervals up to and including 4 hours.

The spores of the molds, grown on Sabouraud's agar slants for 24 days, were removed with saline-gelatin (0.5%) solution and irradiated in the latter medium. Immediately after radiation, dilutions of the specimens were made and plating carried out in Sabouraud's maltose agar. Colony counts were made after the plates had been incubated at room temperature (24-26°C) for 5 days. Table XVI presents the results of this experiment.

TABLE XVI

EFFECTS OF IRRADIATION ON MOLDS

Time of Irradiation hours	A. niger	Per cent Reduction	P. notatum	Per cent Reduction
0 (unirradiated)	45,000,000	0	11,250,000	0
1	125,000	99.66	15,000	99.86
2	5,000	99.98	125	99.99
3	25	99.99	0	100.0
4	0	100.0	0	100.0

i) Experiment No. 9. The Effect of Gamma Radiation (Cobalt-60) on Sporeforming Bacteria, Bacillus subtilis and a Gram-Positive Sporulating Bacillus Isolated from Milk (M-S)*, with Short Periods of Irradiation.

Experiments No. 6 and 7 present the results of cobalt-60 irradiation upon the sporeforming bacilli, B. subtilis and the organism M-S that was

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* See footnote on page 26.

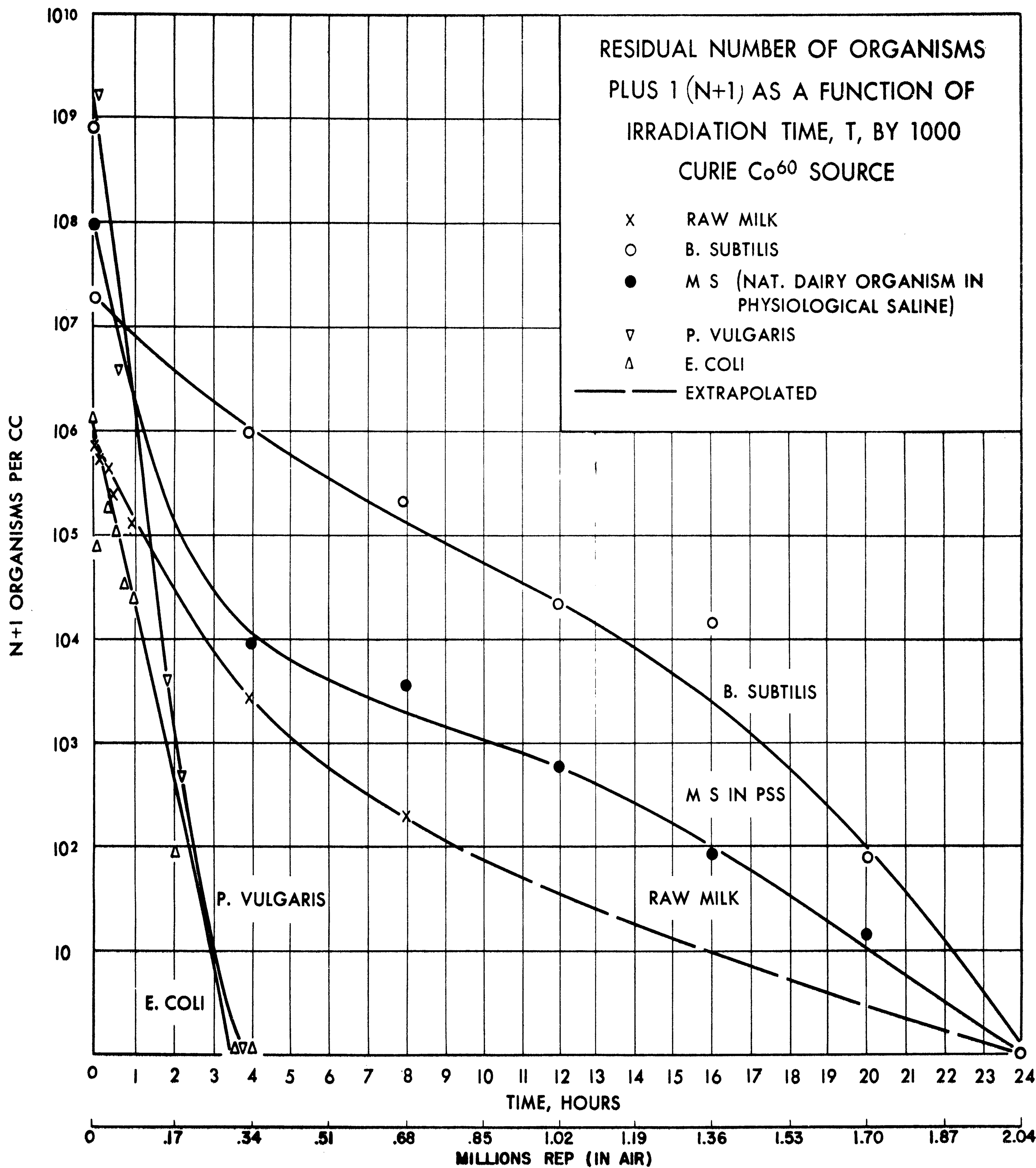

Fig. 11. Risidual Number of Organisms plus 1(N + 1) as a Function of Irradiation Time, T, by 1000 Curie Cobalt-60 Source

isolated originally from milk. In the latter studies a significant reduction in numbers of total bacteria and their spores was noted when samples were first removed at the 4-hour period. The present experiment was carried out to determine the effects of the radiation on the same organisms when the latter were exposed for shorter periods of time. These time intervals of selecting specimens ranged from 15 minutes through 4-hours.

In addition to irradiating the M-S culture suspended in saline-gelatin solution, suspension of the organism was also prepared in evaporated (sterile) milk which was reconstituted to its original concentration with an equal amount of distilled water. The results of this investigation are given in Table XVII.

TABLE XVII

EFFECT OF SHORT PERIODS OF IRRADIATION ON SPOREFORMING BACTERIA

Time of Irradiation	M-S in Milk	% Red.	M-S in saline-gelatin	% Red.	B. subtilis in saline-gelatin	% Red.
0	24,402,000,000	0	7,406,000,000	0	232,000,000	0
15 min	23,600,000	99.90	67,500,000	99.5	131,000,000	43.55
30 min	7,700,000	99.91	7,500,000	99.95	57,000,000	75.44
1 hr	1,600,000	99.99+	2,100,000	99.99+	34,000,000	85.11
2 hr	427,000	99.99+	621,000	99.99+	22,800,000	90.20
3 hr	292,000	99.99+	432,000	99.99+	18,400,000	91.87
4 hr	217,000	99.99+	237,000	99.99+	9,400,000	95.95

j) Experiment No. 10. The Effect of Gamma Radiation (Cobalt-60) on Spore Suspensions of Bacillus subtilis and a Gram-Positive Sporulating Bacillus Isolated from Milk (M-S)*

In the previous studies carried out on the effects of cobalt-60 radiation on the sporeforming bacilli (Experiments No. 6, 7, and 9), no attempt was made to provide a spore suspension of the organisms in the apparent absence of vegetation forms of the respective organism. Conceivable, therefore, much of the initial reduction in number of organisms in the samples may be attributed to the effects of irradiation on the vegetative forms of bacteria, with a subsequent "leveling off" in number which might be due to the resistance of the spores to gamma irradiation. This latter point was the subject of the following experiment. Some 16-day old cultures of B. subtilis and M-S bacteria, grown on beef extract agar, were washed from slants with saline-gelatin solution. The suspensions were then heated in a water bath at 70°C for 30 minutes to destroy the vegetative forms of the organisms. The latter samples were subjected to gamma irradiation for a total period of 24 hours, during which samples of the treated suspensions were removed at intervals of 1 hour and 45 minutes, and again at 4 hours. Dilutions, plating, and

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* See footnote, page 26.

colony counts were made as indicated in Experiment No. 1. The results of this study are presented in Table XVIII.

TABLE XVIII

EFFECT OF LONG PERIODS OF IRRADIATION ON SPOREFORMING BACTERIA

Time of Irradiation	B. subtilis	Per cent Reduction	M-S	Per cent Reduction
0 (unirradiated)	27,500,000	0	1,000,000	0
1 hr, 45 min	3,900,000	49.31	220,000	78.91
4 hrs	890,000	96.83	17,000	96.62
24 hrs	0	100.0	0	100.0

k) Experiment No. 11. The Effects of Gamma Radiation From Cobalt-60 and X-rays upon Yeast (Saccharomyces cerevisiae)*

A strain of yeast (Saccharomyces cerevisiae) was grown on Sabouraud's Maltose agar slants at 37°C for 48 hours. The culture was removed from the surface of the slant in saline-gelatin (0.5%) solution and the suspension centrifuged. The supernatant liquid was removed and discarded. The packed cells were resuspended in saline-gelatin and again centrifuged. After removal of the supernatant fluid the cells were again suspended in fresh saline-gelatin solution, and this material was used in the irradiation studies. Control unirradiated samples were kept at room temperature until completion of the irradiation experiments, at which time all the test samples were diluted and plated in Sabouraud's medium. The details of dilution, plating, and counting the colonies are described in Experiment No. 1. The results of this study are presented in Tables XIX and XX.

TABLE XIX

LETHAL EFFECTS OF COBALT-60 ON YEAST CELLS

Time of Irradiation (hrs)	Saccharomyces cerevisiae Counts (colonies per ml)	Per cent Reduction
0)unirradiated)	2,750,000,000	0
1	200,500,000	92.72
2	26,250,000	99.05
3	32,000	99.99+
4	25,000	99.99+

* The studies on the effects of radiations on yeasts were designed and conducted by Miss Gladys Torres, graduate student in the Department of Bacteriology.

TABLE XX

LETHAL EFFECTS OF X-RAYS ON YEAST CELLS

Dosage in Roentgens	Saccharomyces cerevisiae Counts (colonies per ml)	Per cent Reduction
0	3,250,000,000	0
86,000	55,250,000	98.30
132,000	10,000	99.99+
218,000	9,000	99.99+
344,000	5,500	99.99+

Remarks: From the data given in Tables XIX and XX it is evident that the lethal effects from gamma rays emitted from cobalt-60 and x-rays are comparable in degree. While there appear to be some differences in the counts in the initial irradiation samples (1 and 2 hours, respectively), the percentage reduction variations are within the limits of error encountered in the test procedure.

1) <u>Experiment No. 12. The Effect of Gamma Radiation (Cobalt-60) on Yeast (Saccharomyces cerevisiae) with Reference to Age of Culture and Susceptibility to Radiation Action.</u>

There are several references in the literature indicating that the age of cultures of microorganisms plays an important part in the degree of susceptibility of the organisms to irradiation effects. In general, the evidence available suggests that "young" cultures (24-48 hrs) are more susceptible to the lethal effects of x-rays than are "old" cultures of the same organisms. To determine whether or not the same correlation in difference of age of a culture of yeast cells to the effects of irradiation to gamma rays (cobalt-60) may be noted was the aim of the present experiment. The culture used in the study was the same strain of <u>Saccharomyces cerevisiae</u> used in the previous experiment (No. 11). The age of the organism suspension prepared for radiation and unirradiated control was 8 weeks. No attempt was made to prevent the culture medium from desiccating during the incubation at 37°C for the period indicated. Suspension of the organisms in saline-gelatin, washing of the cells, etc., was carried out as indicated in the previous experiment (No. 11). The results of this study are presented in Table XXI. Included in the table for comparison are the data given in Table XIX in which a 48-hour culture of the organism was exposed to the same radiation source.

TABLE XXI

AGE OF YEAST CULTURE VERSUS SUSCEPTIBILITY TO LETHAL EFFECT OF COBALT-60

Time of Irradiation (hrs)	Saccharomyces cerevisiae		Counts (colonies per ml)	
	48-hr culture	% Red.	1,344*-hr culture	% Red.
0 (unirradiated)	2,750,000,000	0	42,250,000	0
1	200,000,000	92.72	12,000	99.99+
2	26,250,000	99.05	0	100.0
3	32,000	99.99+	0	100.0
4	25,000	99.99+	0	100.0

Remarks: Examination of the data given in Table XXI reveals that, contrary to several published reports on the resistance of "old" cultures of yeast cells to radiation effects, the present results indicate the converse is true; i.e., the "young" cultures of the organisms are more resistant to the effects of gamma rays than are the "old" organism suspensions. The latter is based not only on the initial reduction noted following exposure for 1 hour as reported in per cent reduction, but also on the analysis of the unirradiated control; the first counts at the 1-hour period will indicate the far greater mortality rate of the "old" sample as compared to the "young" (48-hour) culture.

3. The Effects of Radiation on Vitamins Essential for the Nutrition of Microbial Growth

m) Experiment No. 13. The Effect of Gamma Radiation (Cobalt-60) on Niacin (Nicotinic Acid)

A study was made to determine the effects of gamma radiation (cobalt-60) on one of the vitamins that is essential as a growth factor for Lactobacillus arabinosus. The microbiological assay was performed on the irradiated and unirradiated (control) medium using the L. arabinosus strain No. 17-5.

Methods: A reference stock solution of niacin, previously dried to a constant weight in a desiccator, was prepared in 500 ml of absolute alcohol. This stock solution was kept at 5°C when not in use.

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* 8-week old culture.

The culture used in this study was obtained from the American Type Culture Collection, Washington, D. C. The organism was maintained in a "stab" agar culture of Difco Micro-assay agar. Transplants of the stock culture were made at weekly intervals and maintained at 5°C after an initial incubation at 37°C for 24 hours. For assay procedures, transplants were made from the "stab" culture to 10 ml of Difco Micro-Inoculum Broth. The latter cultures were incubated at 37°C for no more than 24 hours, after which the suspension was centrifuged under aseptic conditions. The supernatant broth was discarded and the packed cells resuspended in 10 ml saline (0.85° NaCl) solution. One drop of this suspension was added to each assay tube containing the vitamin-deficient medium and varying amounts of the supplement, niacin substrate. The suspension of organisms was delivered to the assay tubes with a sterile tuberculin syringe and a No. 19 gauge needle.

The dehydrated Difco Niacin Assay Medium used in this study is described on page 11.

The dehydrated medium was reconstituted in the amount of 75 grams to 1000 ml of distilled water by heating just to boiling and then distributing 5-ml amounts in test tubes. The medium was sterilized by autoclaving for no more than 15 minutes at 15 pounds (121°C) steam pressure.

Irradiation: For the irradiation study, 10 ml of the niacin stock solution was added to a 15 x 120-mm screw-capped pyrex test tube (sterile) and the solution exposed to cobalt-60 for 24 hours. A similar sample was maintained in the laboratory at room temperature as a control. The pH of the solutions was 5.1. Microbiological assays on the solutions were performed as soon as possible after the irradiated sample was removed from the cobalt vault.

Microbiological Assay for Niacin: Both the irradiated and unirradiated controls were diluted 1:500 with sterile distilled water in sterile 500-ml volumetric flasks. Amounts equivalent to the following concentration of niacin were placed aseptically in duplicate tubes containing 5 ml of the niacin-deficient assay medium: 0.00, 0.05, 0.10, 0.20, and 0.30 gamma. The volume of liquid in the tubes was then brought up to 10 ml with sterile distilled water. After thoroughly shaking the solution in the tubes, one drop of the culture of *L. arabinosus* was added to each as indicated earlier in this test.

Turbidimetric measurements were made on the solutions after 20 hours incubation at 37°C. A photoelectric colorimeter (Lumetron, Model G Photo Volt Company, New York) with the No. 530 filter was used for the latter procedure. Two blanks were used, one containing 5 ml of niacin assay medium and 5 ml of distilled water but no bacteria inoculum, and the other prepared as before but inoculated with the test organism and incubated as indicated above. The noninoculated control also served as a control on the aseptic technique that is necessary to carry out the microbiological assay of vitamin solutions. Table XXII presents the results of the preliminary test on the effects of cobalt-60 on niacin.

TABLE XXII

THE EFFECTS OF COBALT-60 IRRADIATION ON NIACIN (24 HOURS EXPOSURE)

Original Amount of Niacin Added	Assay Concentration of Niacin in Gammas	
	Nonirradiated	Irradiated
Control (no niacin)	0.00	0.00
0.050	0.058	0.048
0.100	0.100	0.083
0.200	0.200	0.125
0.300	0.300	0.300

Results: The galvanometer readings were converted into optical density by the relation L = 2 log G. A standard curve was drawn using the values obtained from the assay of the nonirradiated sample of niacin. Values of the various concentration levels from the irradiated sample were then read from the standard curve.

Within the limits of experimental error of this test procedure, the data in Table XXII indicate that exposure of the niacin solution to cobalt-60 for a period of 24 hours will not appreciably alter the vitamin insofar as its growth-promoting ability for *L. arabinosus* is concerned.

Additional studies are contemplated in which other vitamins, in solution and in the dry state, will be exposed to gamma and x-rays.

n) Experiment No. 14. The Effect of Gamma Radiation (Cobalt-60) on a Chemically Defined Medium which Supports the Growth of *Lactobacillus arabinosus*.

The data given in the preceding experiment (No. 13) indicate that exposure of an aqueous solution of niacin to 24 hours irradiation by cobalt-60 did not destroy the growth-promoting component of the vitamin for *L. arabinosus*. Since there are twenty ingredients, including vitamins, etc., in the basal niacin-deficient medium (Difco), as listed in the previous experiment, it appeared desirable to determine whether or not irradiation of the latter would result in the inactivation of some of these components in the formula, or the production of toxic substances there from which would inhibit the growth of *L. arabinosus* in the presence of its essential vitamin, namely niacin.

Method: For this experiment, 15 ml of double-strength niacin medium (Difco--see preceding experiment) were irradiated, with sufficient niacin added, for 6 hours with cobalt-60. The amount of niacin added to the medium provided a concentration of 0.1 gamma which was to be tested in the final dilution in each assay tube. Following irradiation, 5 ml of the solution

was added to each assay tube and the latter volume diluted to 10 ml with sterile distilled water. Thus, the concentration of the medium was the same as in the determination of niacin in the preceding experiment but in this study the concentration of niacin per tube was 0.1 gamma.

Each tube of medium was inoculated with one drop of a 20-hour washed culture of L. arabinosus and allowed to incubate at 37°C for 20 hours. Turbidimetric measurements were made using appropriate blank controls. The optical densities of the control and irradiated samples are given in Table XXIII. Duplicate sets of tubes were prepared in each instance (Tubes 1 and 2).

TABLE XXIII

THE EFFECT OF COBALT-60 ON A BACTERIOLOGICAL MEDIUM

Tube No.	Nonirradiated	Irradiated for 6 hours
1	0.143	0.131
2	0.137	0.125

Results: From the data given in Table XXIII it is evident that irradiation of the Niacin Assay Medium to which was added the essential vitamin, niacin, for the propagation of L. arabinosus, did not result in any apparent change in the medium that could be detected in the microbiological assay or growth of the organism.

4. The Effects of Gamma Radiation on Enzyme Systems

o) Experiment No. 15. The Effect of Gamma Radiation (Cobalt-60) on Various Proteolytic Enzyme Systems.

Methods: The following are the methods that were employed in the preparation of the enzyme suspensions for irradiation and the determination of their activity:

(1) Pepsin: The pepsin used in the experiments was a recrystallized product of porcine origin obtained from Armour and Company. For irradiation, 40 mg were dissolved in distilled water adjusted to a pH of 1.1 with hydrochloric acid. Then 20 ml of this suspension were irradiated in 12 x 60-mm pyrex glass test tubes. At indicated time intervals 3 ml samples were withdrawn. At unirradiated control containing the same amount of enzyme suspension was kept for the entire period of irradiation. Proteolytic activity

To 1 ml of the supernatant liquid 1 ml of 0.0025M copper sulfate, 8 ml of 0.5M sodium hydroxide, and 3 ml of Folin-Ciocalteau reagent diluted 1:2 were added. The per cent transmission was read in a photoelectric colorimeter using a No. 650 filter and appropriate blank.

(3) Chymotrypsin: The chymotrypsin used in this experiment was a recrystallized product of bovine origin obtained from Armour and Company; 20 mg of the enzyme was dissolved in 50 ml of distilled water adjusted to a pH of 2.7 with hydrochloric acid, and 4 ml of this solution was irradiated in 5 x 35-mm test tubes. Individual tubes were withdrawn at the indicated time intervals. A control was kept at room temperature during the time of irradiation.

The activity of the chymotrypsin was determined after the last irradiation. The casein method was also used for the assay of the latter enzyme.

(4) Papain: For this experiment 500 mg of papain (Merck) was dissolved in 100 ml of distilled water and the solution filtered. In order to activate the enzyme, 10 drops of 2N sodium cyanide were added to 1 ml of the enzyme solution. After standing at room temperature for 10 minutes, 18.9 ml of distilled water was added to the latter. The solution was further diluted by addition of 10 ml of distilled water containing 5 drops of 2N sodium cyanide. The pH of the enzyme solution was 5.7.

For irradiation, 5 ml was placed in 5 x 35-mm test tubes and individual tubes were withdrawn at the indicated time intervals. A control tube containing unirradiated enzyme solution was kept at room temperature during the period of irradiation of the test sample.

Proteolytic activity was determined after the last radiation. The method used for the determination was the same as that used for trypsin. The results of this study are presented in Tables XXIV and XXV.

TABLE XXIV

EFFECTS OF GAMMA IRRADIATION ON ACTIVITY OF PEPSIN

Time of Irradiation	Milligrams of Tyrosine
0	0.0860
51 min	0.0720
1 hr, 46 min	0.0535
2 hr, 26 min	0.02875
3 hr, 41 min	0.00240
4 hr, 28 min	0.00076

TABLE XXV

EFFECT OF GAMMA IRRADIATION ON ACTIVITY OF TRYPSIN, CHYMOTRYPSIN, AND PAPAIN

Time of Irradiation (hrs)	Trypsin	Milligrams of Tyrosine Chymotrypsin	Papain
0	0.0945	0.0945	0.0635
1/2	------	0.0835	0.04815
1	0.0762	0.0505	0.0275
2	0.017	0.00345	0.01625
3	0.0040	0.00225	0.019
4	0.0035	0.00075	0.017

Results of Studies on Effects of Cobalt-60 in Enzyme Systems: The per cent transmission of the various solutions was converted to optical density (L) by the formula, $L = 2 - \log G$. Tyrosine values were read from a standard curve prepared previously, using known concentrations of tyrosine. The amount of tyrosine liberated by the hydrolysis of the proteins by the enzymes was taken as an index of the activity of the enzymes. The results of the various experiments are expressed as milligrams of tyrosine released. Tyrosine values were plotted against time of irradiation and are presented in Fig. 12.

p) Experiment No. 16. Effect of Concentration, Cystine and Sodium Pyrovate on Enzymatic Activity Following Irradiation with Gamma Radiation (Cobalt-60).

The following set of experiments demonstrates the protection offered by alteration of radiation.

In one case various concentrations of enzymes were irradiated. In those instances where extreme conditions were used the enzyme solution had to be diluted after radiation in order to conduct the assay. Conditions of radiation and assay procedure are as outlined previously.

In testing the activity in the presence of cystine and sodium pyruvate, 1% solutions were mixed with an equal quantity of enzyme solution, which gave a final concentration of 0.5% of the chemical during radiation. Concentration of enzymes used during radiation is indicated for the individual experiments, Tables XXVI to XXIX. Figs. 13 to 16 present plots of this data.

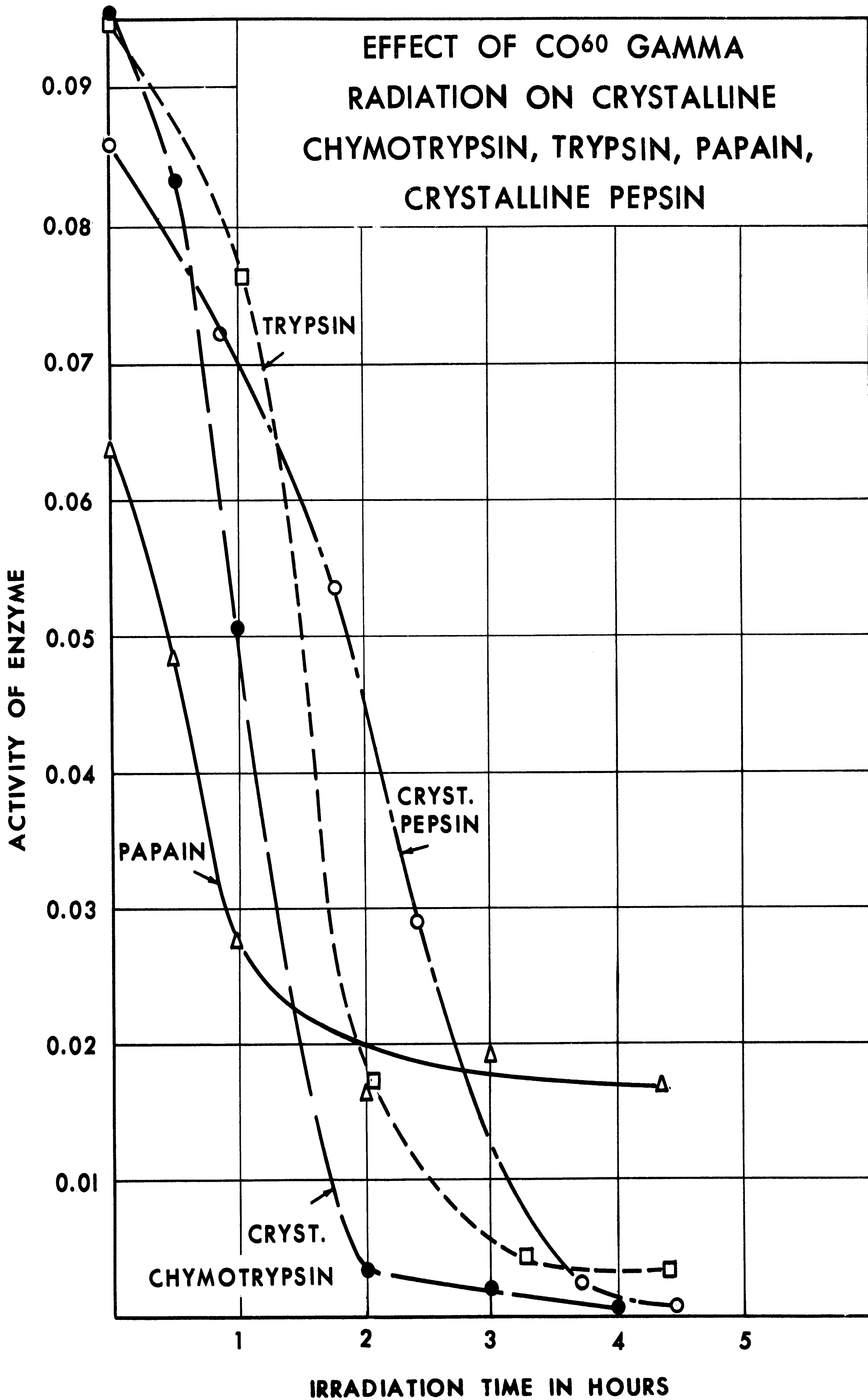


Fig. 12. Effect of Co^{60} Gamma Radiation on Crystalline Enzymes

TABLE XXVI

PROTECTIVE EFFECT OF, CYSTINE AND SODIUM PYRUVATE
ON PEPSIN FOLLOWING IRRADIATION WITH COBALT-60

Time of Radiation	Dose Rep. in Air	H_2O	Mg/ml of Tyrosine	
			0.5% Sodium Pyruvate	0.5% Cystine
0	0	0.30	0.3	0.30
1	80,000	0.181	0.25	0.30
2	160,000	0.105	0.181	0.30
3	240,000	0.016	0.090	0.285
4	320,000	0.00	0.044	0.256

Conc. pepsin = 0.25 Mg/ml
pH of solution = 1.1
4 ml radiated in 10 x 70-mm test tubes
Temp.: 25°-26°C

TABLE XXVII

PROTECTIVE EFFECT OF ENZYME CONCENTRATION
ON PEPSIN FOLLOWING IRRADIATION WITH COBALT-60

Radiation Time (Hrs)	Dose Rep. in Air	mg/ml of Tyrosine		
		10 mg/ml	1 mg/ml	0.10 mg/ml
0	0	0.30	0.158	0.012
1	80,000	0.30	0.095 (?)	0
2	160,000	0.30	0.085	0
3	240,000	0.30	0.058	0

pH of solution = pH 1.1
4 ml in 10 x 70-mm tubes Radiated
Temp. 25°-26°C

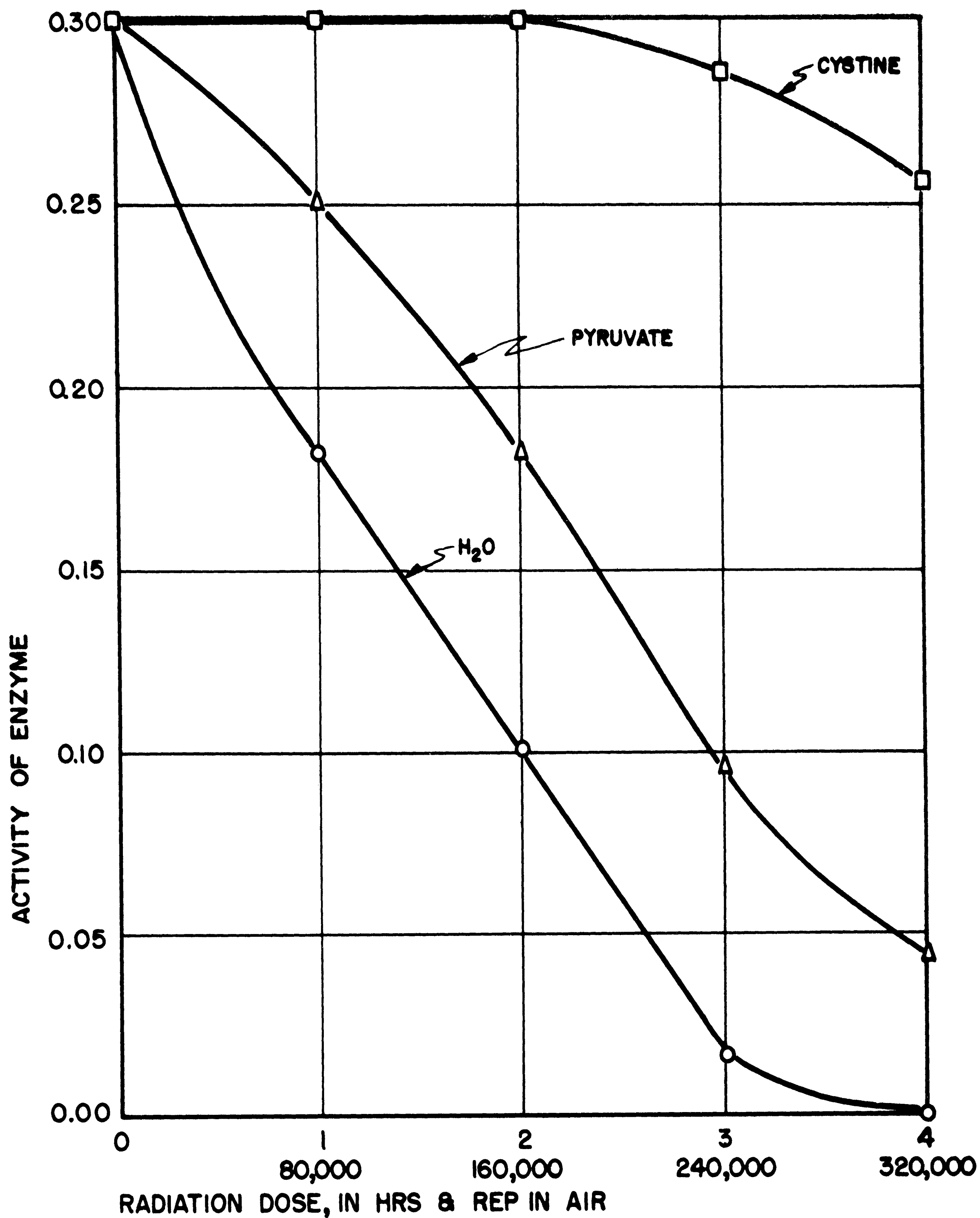


FIG. 13. PROTECTIVE EFFECT OF *l*-CYSTINE AND SODIUM PYRUVATE ON PEPSIN DURING IRRADIATION.

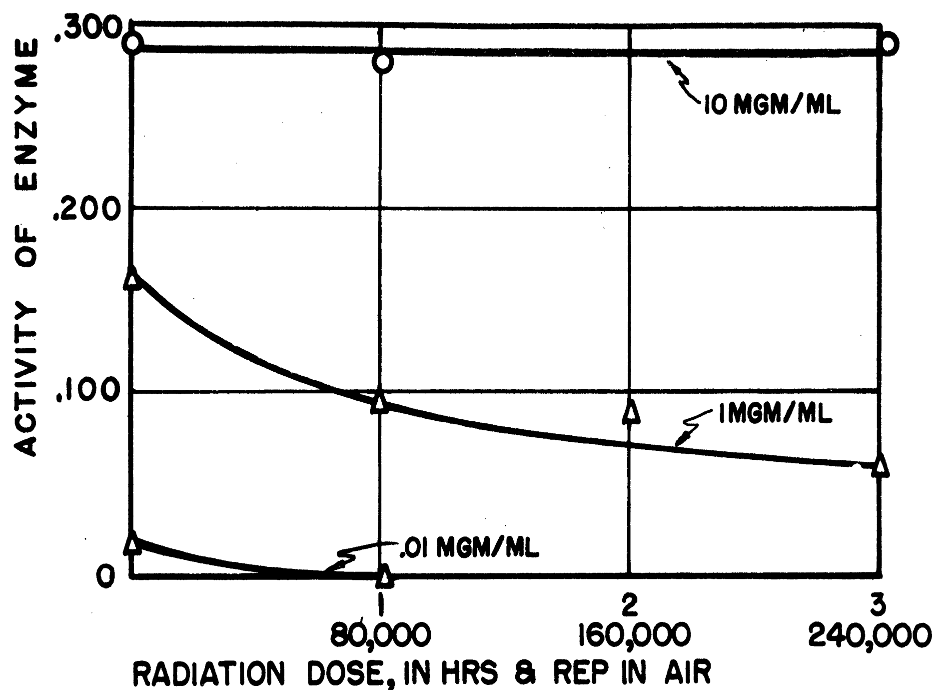


FIG. 14. EFFECT OF ENZYME CONCENTRATION ON PEPTIC ACTIVITY FOLLOWING IRRADIATION WITH GAMMA RAYS FROM COBALT-60

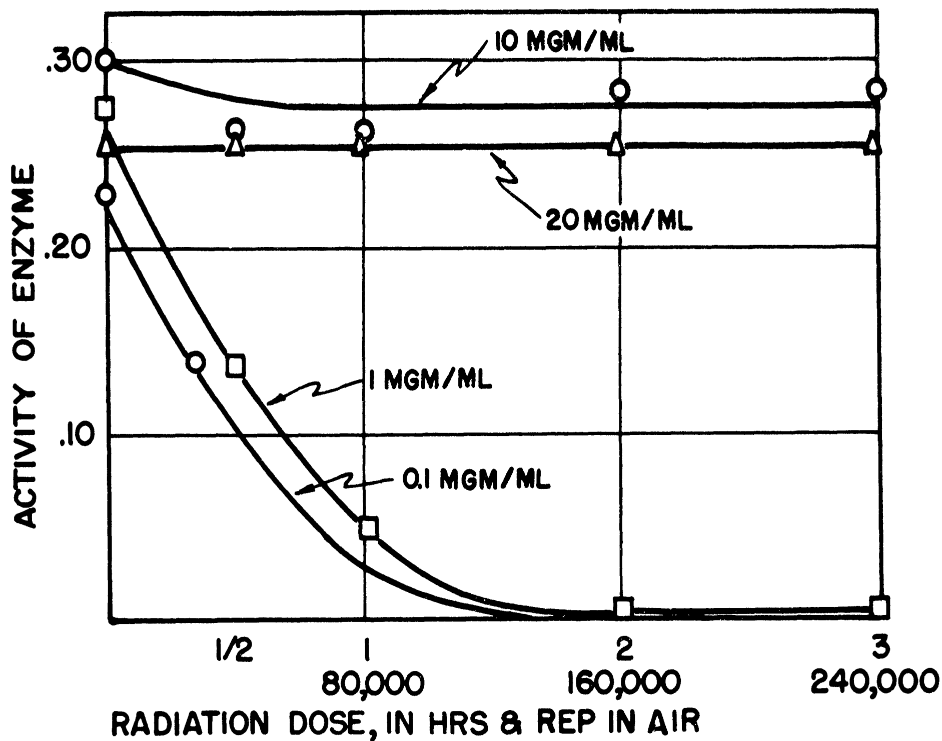
TABLE XXVIII

PROTECTIVE EFFECT OF ENZYME CONCENTRATION ON TRYPSIN DURING RADIATION

Time (Hrs)	Dose (Rep. in Air)	Mgm/ml of tyrosine 50 mg/ml	Mgm/ml of tyrosine 0.5 mg/ml	Mgm/ml of tyrosine 0.05 mg/ml
0	0	.167	.275	.105
1	80,000	.147	.256	.005
2	160,000	.160	.14	.005
3	240,000	.153	.04	.003
4	320,000	.147	.015	.003

Time (Hrs)	Dose (Rep. in Air)	Mgm/ml of tyrosine 20 mg/ml	Mgm/ml of tyrosine 10 mg/ml	Mgm/ml of tyrosine 1 mg/ml	Mgm/ml of tyrosine 0.1 mg/ml
0	0	.254	.30	.275	.230
1/2	40,000	.254	.263	.137	----
1	80,000	.254	.256	.048	.141
2	160,000	.254	.285	.005	.005
3	240,000	.254	.285	.005	.004

4 ml radiated per tube 10 x 75 mm
pH 2.4
Temp 25°-26°C



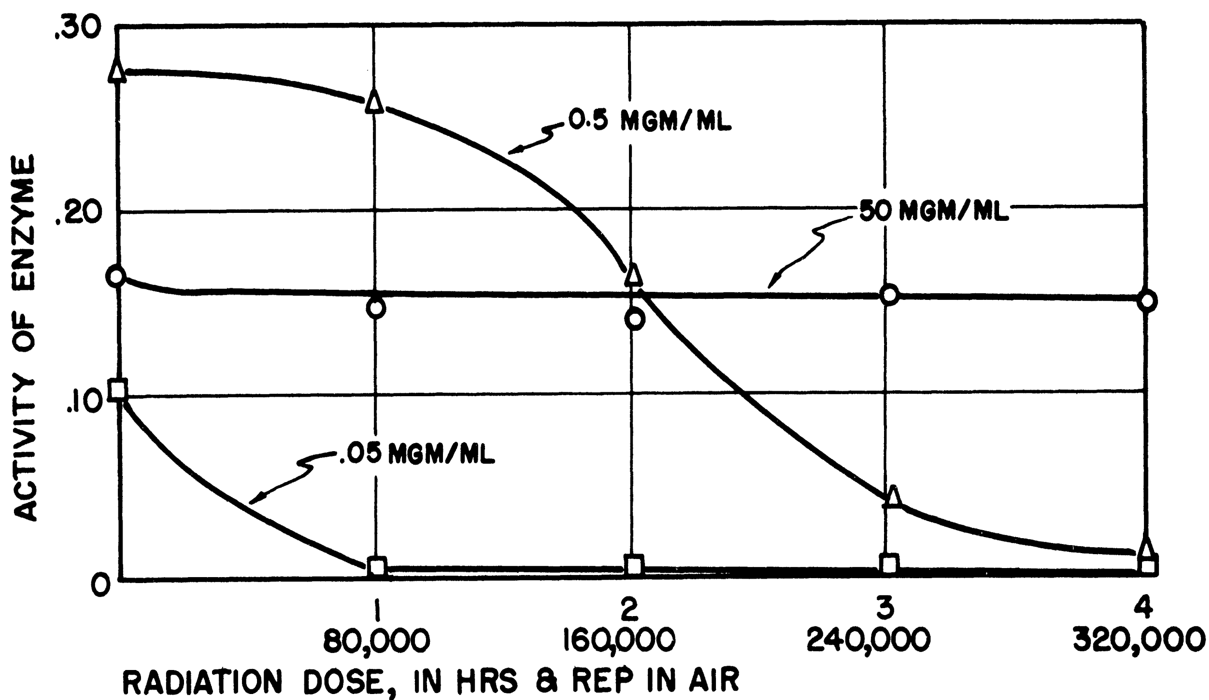


FIG. 15. EFFECT OF CONCENTRATION ON ACTIVITY OF TRYPSIN

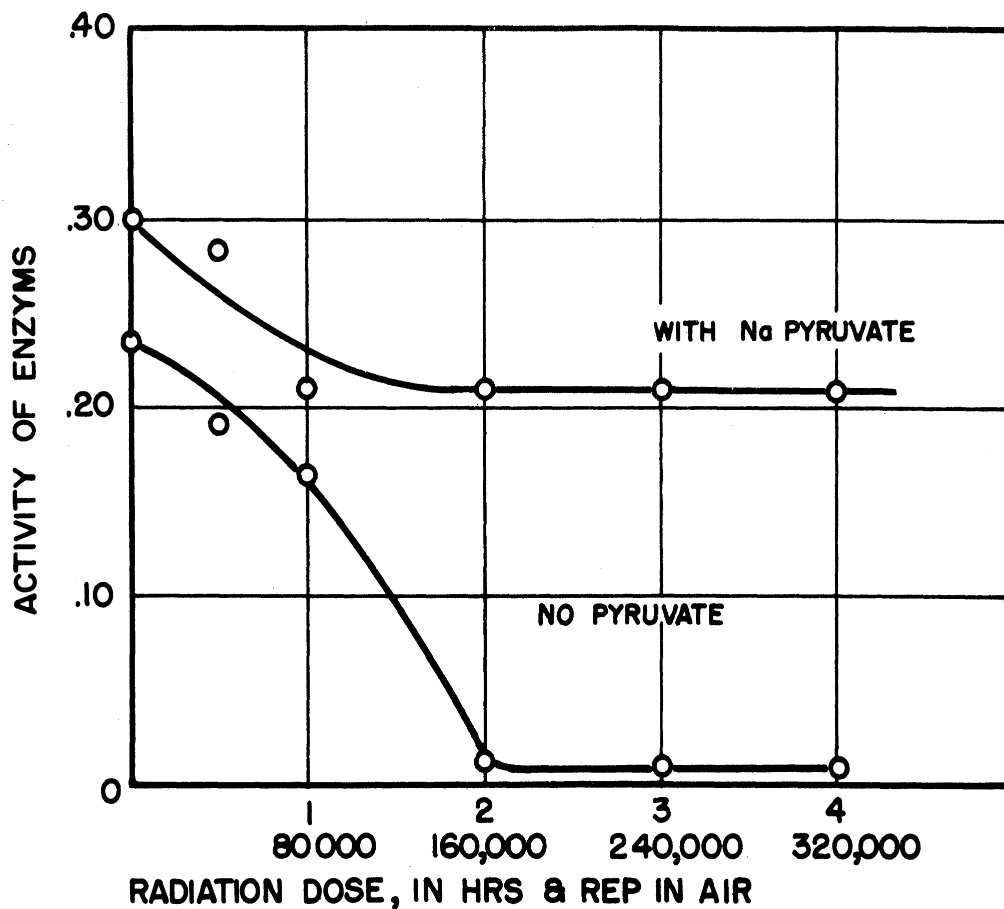


FIG. 16. PROTECTION EFFECT OF 0.5 PER CENT SODIUM PYRUVATE ON TRYPSIN DURING IRRADIATION

TABLE XXIX

PROTECTIVE EFFECT OF SODIUM PYRUVATE
ON TRYPTIC ACTIVITY DURING RADIATION

Time (Hrs)	Dose (Rep. in Air)	Mgm/ml of tryosine	
		H_2O	Na Pyruvate 0.5%
0	0	0.23	0.30
1/2	40,000	0.185	0.256
1	80,000	0.167	0.212
2	160,000	0.019	0.212
3	240,000	0.01	0.212
4	320,000	0.01	0.212

Conc. enzyme = 0.2 mgm/ml
pH 2.54

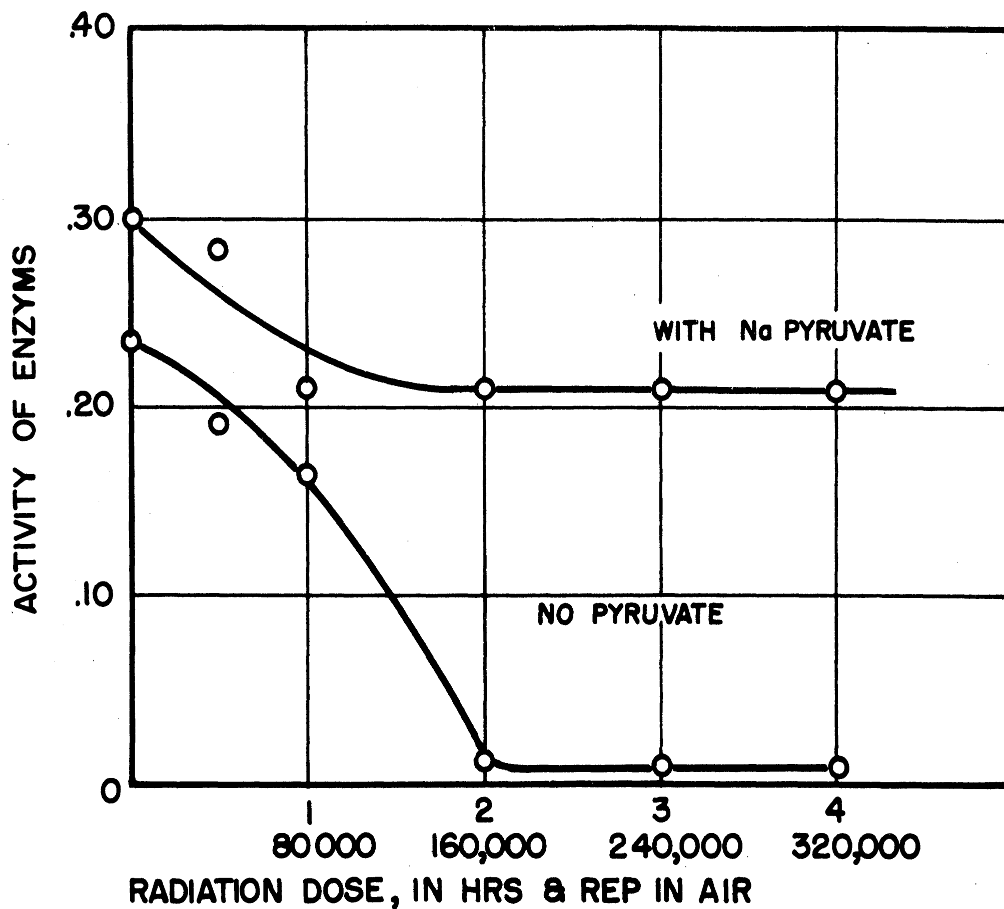


FIG. 16. PROTECTION EFFECT OF 0.5 PER CENT SODIUM PYRUVATE ON TRYPSIN DURING IRRADIATION

TABLE XXIX

PROTECTIVE EFFECT OF SODIUM PYRUVATE ON TRYPTIC ACTIVITY DURING RADIATION

Time (Hrs)	Dose (Rep. in Air)	Mgm/ml of tryosine	
		H_2O	Na Pyruvate 0.5%
0	0	0.23	0.30
1/2	40,000	0.185	0.256
1	80,000	0.167	0.212
2	160,000	0.019	0.212
3	240,000	0.01	0.212
4	320,000	0.01	0.212

Conc. enzyme = 0.2 mgm/ml
pH 2.54

q) Experiment No. 17. Effect of Gamma Radiation (Cobalt-60) on the Phosphatase Activity of Raw Milk.

Phosphatase, an enzyme present in raw milk, is used as an index of pasteurization because it is readily inactivated by heat. In order to test what effect, if any, gamma radiation from cobalt-60 had on phosphatase activity, raw milk obtained from a local dairy was irradiated at various time intervals.

Phosphatase activity was measured by its action on disodium phenyl phosphate. The amount of phenol liberated was measured photometerically.

Procedure:[65] The buffer-substrate was made by dissolving 1.09 g disodium phenyl phosphate and 11.54 g sodium vernol in 1 liter of distilled water saturated with chloroform. This substrate was kept at 5°C when not in use. For the test 10 ml of substrate was added to 16 x 120-mm test tubes and one drop of chloroform was added to keep down bacterial growth. To each of duplicate tubes, 0.5 ml of raw milk was added, after which the tubes were incubated at 37.5°C for 18-24 hours. They were then removed and cooled at room temperature. To the tubes were added 4.5 ml of Folin Ciocalteau reagent diluted 1:2 and allowed to stand for three minutes. The suspension was filtered using a No. 3 Whatman filter, to 5-ml amounts of filtrate was added 1 ml of anhydrous 14% $NaCO_3$ and the tubes placed in a boiling water bath for 15 minutes. After cooling and filtering, the color that developed was read in a photoelectric colorimeter (Lumetron-Model 400-G) using a No. 630 filter.

Concentration of phenol was obtained from standard curve prepared previously using known concentrations of phenol.

The results are expressed as mg per ml of phenol.

Irradiation: For irradiation, 2 ml of milk was suspended in 10 x 95 mm tubes and exposed for the various time intervals indicated. Samples were irradiated at room temperature. A control was maintained at room temperature during irradiation of the samples.

For dilutions, raw milk was mixed with indicated amounts of sterile distilled water and 10 ml were radiated in 60 x 150-mm test tubes for 19 hours. The results of this study are presented in Tables XXX and XXXI.

TABLE XXX

PHOSPHATASE ACTIVITY IN RAW MILK AFTER IRRADIATION

Radiation Time	Dose (Rep. in Air)	Phenol mg/ml
0	0	0.55
1.75	140,000	0.55

TABLE XXX (Cont.)

Radiation Time	Dose (Rep. in air)	Phenol mg/ml
4.50	360,000	0.48
11.25	900,000	0.51
24.0	1,920,000	0.43

TABLE XXXI

PHOSPHATASE ACTIVITY IN VARIOUS DILUTIONS OF MILK AFTER IRRADIATION

Sample*	Dose (Rep. in air)	Phenol mg/ml Dilutions of Milk**			
		0	1:10	1:100	1:1000
Control No. 1	0	0.354	0.374	0.134	0.059
Control No. 2	0	0.398	0.374	0.148	0.059
Irradiated No. 1	1,920,000	0.484	0.364	0.148	0.050
Irradiated No. 2	1,920,000	0.498	0.364	0.096	0.059

Results: From the data given in the tables it will be noted that the phosphatase activity in raw milk is not affected to any appreciable extent even after 24 hours of radiation. Diluting milk out to 1:1000 did not increase the sensitivity of phosphate to radiation.

5. The Effects of Gamma Radiation on Various Microorganisms

r) Experiment No. 18. The Effect of Gamma Radiation (Cobalt-60) on Trichomonas foetus.

For this experiment 1-ml amounts of a 4-day culture of Trichomonas foetus, suspended in the fluid part of Schneider's media,[66] were irradiated in 10 x 75-mm tubes for the indicated time intervals. Motility and numbers were ascertained by "hanging-drop" preparations before and after irradiation. Also, 0.1-ml amounts were inoculated into duplicate tubes of Schneider's medium after radiation and checked for growth after 4-5 days at 37.5°C by the "hanging-drop" method. All irradiations were carried out at room temperature (25-26°C). An appropriate control, which did not receive any radiation but otherwise was treated in the same manner as the radiated sample, was also used. The results of this study are given in Table XXXII.

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* 10 ml amounts irradiated

**Diluted with sterile distilled water

TABLE XXXII

EFFECT OF GAMMA RADIATION (COBALT-60) ON TRICHOMONAS FOETUS

Radiation Time	Radiation Dose (Rep. in air)	Motility		Growth 4 days, 37.5°C	Amount Irradiated
		Before Radiation	After Radiation		
0	0	+	+	+	
1	80,000	+	+	+	
3	240,000	+	+	+	1 ml
5	400,000	+	+	0	
7	560,000	+	+	0	
0 min	0	+	+	+	
- min		+	+	+	
15 min	20,000	+	+	+	0.75 ml
30 min	40,000	+	+	+	
60 min	80,000	+	+	+	
0 hrs	0	+	+	+	
1 hr	80,000	+	+	+	
4 hrs	320,000	+	+	n.g.	1 ml
5 hrs	400,000	+	+	(-) in one (-) in one	

Results: From the data given in the table it will be noted that growth of the Trichomonas is prevented in 5 hours (400,000 Rep. in air) but not in 3 hours (240,000 Rep. in air) of irradiation. Another experiment indicated that growth is prevented in 4 hours (320,000 Rep. in air) but not in 3 hours (240,000 Rep. in air). Motility is not immediately affected by irradiation except in the higher doses. The cells move more slowly and there is evidence of cell destruction. No attempt was made to estimate the number of cells which were inactivated or destroyed.

s) Experiment No. 19. The Effect of Gamma Radiation (Cobalt-60) on Viruses.*

Several references in the introductory chapter of this report indicated the effects of radiation on viruses. Hodes, Webster, and Lavin[3] were able to inactivate rabies virus with ultraviolet radiation and yet retain its immunizing properties more effectively than chloroform or phenol-treated virus. Huber and his associates[28,40] reported on the effects of a high

* This study was carried out in collaboration with R. D. Francis, graduate student in the Department of Bacteriology of the Medical School.

intensity electron burst on a number of viruses and also demonstrated that an effective immunizing rabies virus could be prepared by the use of the capacitron. Their studies indicated that this form of radiation was generally more effective in destroying the infectivity of several viruses than were radiations from beta, gamma, or x-rays. There is a general agreement that viruses are more resistant to the lethal effects of radiations than are bacteria, molds, yeasts, and protozoa.

Experimental: Psittacosis (strain 6BC) and Human pneumonitis (strain SF) viruses were selected for the present study. A freshly harvested embryonic chick allantoic fluid preparation of each virus, visibly free of particulate matter, was diluted 1:2 with sterile physiological saline solution and divided into two equal portions. One portion was exposed to gamma radiation emitted from the cobalt-60 source. The other half served as the control and was held at room temperature, equivalent to that in the cobalt vault. All the virus material was maintained in cotton-plugged pyrex test tubes. At various time intervals aliquot amounts of the irradiated, as well as the control, virus suspensions were removed from the tubes and stored immediately at -46°C until tests for inactivation could be made.

Tests for complete inactivation of virus and infectivity titration were conducted by intracerebral inoculation of 10-14 gram albino mice. Six to ten mice were used in testing each specimen of irradiated virus. Infectivity titrations were performed by preparing a series of tenfold dilutions of virus in veal infusion broth; six mice being inoculated with each dilution of virus. After the mice were inoculated, they were observed for sign and symptoms of virus infection, time of deaths, and survivals in the irradiated and unirradiated control series. In the latter group the animals invariably died within a period of 5 days following inoculation. The animals surviving the experiment were observed for a period of 10-12 days before being discarded. Table XXXIII presents the results of this study.

TABLE XXXIII

EFFECT OF GAMMA RADIATION (COBALT-60)
ON PSITTACOSIS AND HUMAN PNEUMONITIS VIRUSES

Radiation Time (Hrs)	Radiation Dose (Rep. in air)	Psittacosis		Human Pneumonitis	
		Radiated	Control LD_{50}*	Radiated	Control LD_{50}
0	0	---	$10^{-6.5}$	---	$10^{-5.0}$
1	80,000	Active	---	Active	---
3	240,000	Inactive	---	Inactive	---
6	480,000	Inactive	$10^{-6.6}$	Inactive	$10^{-5.2}$

* LD_{50} = Dilution which will give 50% mortality in animals tested.

Results: From the data given in the table it will be noted that both viruses were completely inactivated after exposure to cobalt-60 for 3 hours or longer. Exposure of the suspensions for 1 hour was insufficient to destroy the same agents. That the inactivation obtained at 3 (240,000 Rep. in air) and 6 hours (480,000 Rep. in air) was due to the specific action of the gamma radiation and not the result of thermo inactivation is indicated by the fact that there was no decrease in the LD_{50} titers of the control specimens, which were also maintained at room temperature during the maximum exposure period employed.

Although the present work is only preliminary nature, it serves to indicate that the virus preparations employed here can be inactivated in a relatively short period of exposure to gamma rays emitted by cobalt-60. Further study will be required to determine if this method of inactivation alters the antigenicity of the viruses under consideration.

t) Experiment No. 20. The Effect of Gamma Radiation (Cobalt-60) on a Virus of Bacteria (Staphylococcus Bacteriophage)

Much of the work on the effect of radiation on bacteriophage (bacterial viruses) has been concerned with the action of radiation on mutagenic or gene changes in the latter particles.[67,68,69] Brasch and Huber[35] found that when bacteriophage were exposed to the capacitron these viruses were destroyed by lower dosages than those required for the destruction or "sterilization" of animal viruses. In the present investigation a broth culture of a Staphylococcus aureus bacteriophage was exposed to gamma radiations from cobalt-60 for varying periods of time to determine the lethal effects of the latter upon this particulate agent.

Experimental: The bacteria-free broth culture of phage was distributed in 2-ml amounts in several cotton-plugged pyrex serological tubes. A control tube was kept at room temperature through the period that the remaining tubes were placed in the cobalt vault. At the termination of the exposure period, all the tubes were stored in the refrigerator and tested the following day for bacteriophage activity.

To test the activity of the several samples of phage, beef extract agar was poured into several sterile Petri dishes. After the medium had solidified, approximately 2 ml of a 1:100 saline dilution of a 24-hour broth culture of S. aureus was placed on the surface of the medium. The suspension was flooded over the entire surface of the agar and the excess removed with a sterile pipette. The plates were then placed at 37°C with the covers of the dishes removed, and the suspension of organisms allowed to dry on the surface of the agar for a period of 30 minutes. When the cultures were returned to the laboratory, the dishes were tilted on the laboratory table by placing one edge of the plates on a section of rubber tubing. One drop of each suspension of treated bacteriophage, as well as the unexposed control, was placed at the upper edge of the agar medium and allowed to flow down the agar surface by gravity. The plates were then incubated at 37°C and the

activity of the bacteriophage on the staphylococcus noted at the end of 24 hours. Fig. 17 presents photographs of the results of this experiment.

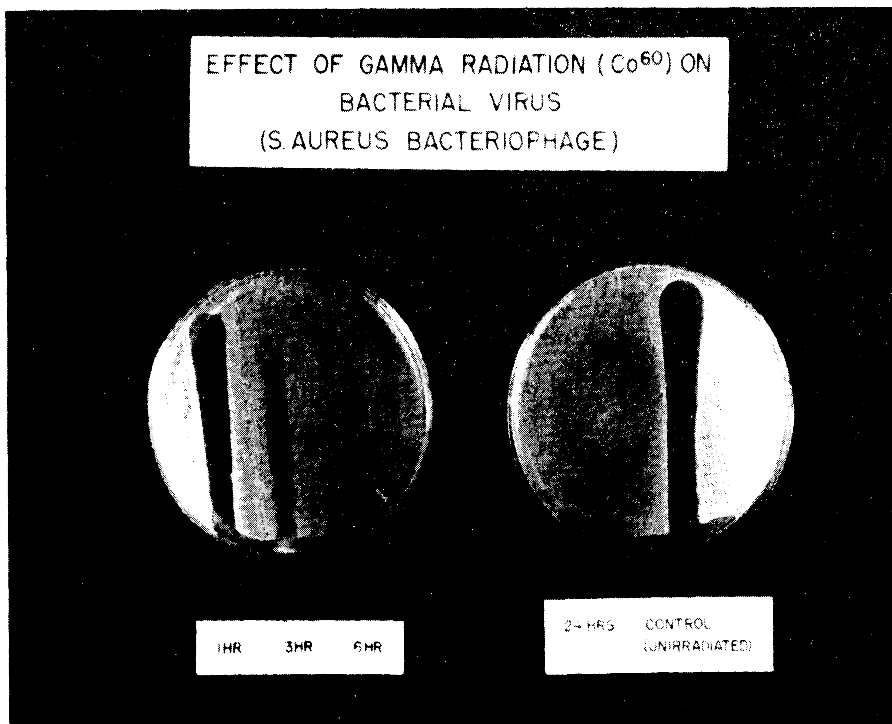

Fig. 17. Photograph of the Effect of Gamma Radiation (Co^{60}) on Bacterial Virus (S. aureus Bacteriophage)

Results: It is evident from the data presented in the photographs of the agar plates that 1-hour (80,000 Rep. in air) exposure of the bacteriophage to cobalt-60 resulted in no detectable difference in the virus as compared to the unirradiated control. It should be stressed, however, that this preliminary test is a very crude technique for detection of phage activity. If a more refined procedure (dilution) is used in subsequent studies, partial destruction of the bacteriophage at the 1-hour (80,000 Rep. in air) period may be detected. The photographs indicate that after 3 hours (240,000 Rep. in air) of Gamma irradiation of the phage, approximately 50 per cent of the particles may have been destroyed, and following 6 to 24 hours (480,000 to 1,920,000 Rep. in air), complete destruction of the bacteriophage can be expected. Subsequent experimentation in this phase of work will include attempts to photoreactivate irradiated bacteriophage.[14,15]

u) Experiment No. 21. The Effect of Gamma Radiation (Cobalt-60) on a Human Strain of Mycobacterium Tuberculosis.

A study was made of the effects of cobalt-60 upon a mouse-virulent strain of *M. tuberculosis* (Olsen strain) which was originally isolated from a human source of clinical tuberculosis. Brief mention has been made in the introductory chapter of this report on the effects of ultraviolet and x-rays on acid-fast bacilli.

Experimental: A suspension of the tubercle organisms was prepared by removing 5 loopfuls of a heavy growth of the organisms from a Petragnani's slant that had been incubated at 37°C for a period of 11 weeks. The organisms were added to a small screw-capped bottle, containing glass beads and 5 ml of sterile saline solution (0.85% NaCl). The contents of the container were shaken vigorously in order to break up the bacterial culture following which an additional 30 ml of saline solution was added to the container. The bacterial suspension was distributed in 5-ml portions into 15-ml vaccine bottles provided with rubber stoppers. A control bottle was maintained at room temperature throughout the period that several of the suspensions were irradiated with gamma rays from cobalt-60. Following the irradiation period, all samples were placed in the refrigerator. A portion of each sample was placed on the surface of duplicate tubes of Petragnani's medium 24 hours after irradiation. The latter were incubated at 37°C for a period of 2 months and the presence or absence of growth of the tubercle bacilli noted.

Two days after the irradiation experiment, 0.1-ml amounts of each suspension, including the control, were injected intravenously by the tail-vein route into each of 5 normal albino mice (this injection series was carried out by Dr. D. W. Smith, postgraduate fellow, and A. A. Grover, graduate student, of the Department of Bacteriology). The animals were kept on a normal Rockland mouse food diet throughout the experiment, i.e. for a period of 53 days, at which time the animals surviving the study were sacrificed by ether anesthesia. One of the control animals died during the second week following injection. However, upon autopsy it was determined that this individual died from some other cause than from the injection of the tubercle organisms. Two additional animals in the control series were found dead on the 45th day of the experiment; however, the conditions of the deceased animals were such (canabalism by the surviving animals in the same cage) that autopsy did not appear advisable. This was also true of 3 animals that died on the 42 and 43rd days of the experiment in the 3-hour (240,000 Rep. in air) irradiation series. Nevertheless, from the animals that were autopsied at the termination of the experiment and the data obtained in the Petragnani's cultures, certain conclusions could be drawn from this preliminary experiment. The results of this study are presented in Table XXXIV.

TABLE XXXIV

THE EFFECTS OF GAMMA RADIATION (COBALT-60) ON M. TUBERCULOSIS (OLSEN STRAIN)

Hrs Irradiation of TB Culture	Radiation Dose (Rep. in Air)	Growth on Petragnani's Medium	Mouse Inoculation Results			
			Animals Inoculated	Animals surviving Test	Gross Path	Acid-fast stains
0	0	Positive	5	2	Pos.	Pos.
1	80,000	Positive	5	5	Pos.	Questionable*
3	240,000	None	5	2*	Neg.	Neg.
6	480,000	None	5	5	Neg.	Neg.
24	1,920,000	None	5	5	Neg.	

* See remarks in the above text.

Results: The control animals which received unirradiated tubercle bacilli showed definite evidence of tuberculosis of the lungs. Slide smears and acid-fast stains of the lungs showed definite evidence of acid-fast bacilli. The animals surviving in the 1-hour (80,000 Rep. in air) irradiated suspension series showed some evidence of gross pathology of the lung tissue; however, diligent search of acid-fast preparations on the same tissue failed to show evidence of tubercle bacilli. The remaining animals in the 3-hour, 6-hour and 24 hour (240,000, 480,000 and 1,920,000 Rep. in air) groups showed no evidence of gross pathology of the lungs or spleen, nor were there any acid-fast bacilli detected in the same smears of these tissues. Observations of Petragnani's slant showed a luxuriant growth of the tubercle bacilli in the unirradiated control and also a good growth, although less in amount than the control, in the 1-hour (80,000 Rep. in air) irradiated suspension. The tubes containing suspensions from the 3-hour, 6-hour, and 24-hour (240,000, 480,000, and 1,900,000 Rep. in air) irradiated series were completely devoid of colonies at the termination of the experiment. Although the suspensions were all inadvertently destroyed during the intervening period of the experiment, thus preventing a study of the possible immunizing properties of the killed vaciines, additional studies on these organisms are contemplated in which this subject will be taken into consideration.

6. The Effect of Gamma Radiation of Toxins, Antisera and Antibiotics

v) Experiment No. 22. The Effect of Gamma Radiation (Cobalt-60) on Tetanus Toxin.*

Tetanus toxin is a labile protein substance and a wide variety of mild chemical and physical agents render it nontoxic for experimental animals. Physical agents found to be effective are mild heating and radioactive materials such as radium; also, ultraviolet light and chlorophyll have been noted to have an inhibiting action on the toxin. Efrati[70] studied the effects of x-rays on the same system. Larson, Evans, and Nelson[71] and Vincent[72] described the action of surface-active soaps on the toxin. The activity of various oxidizing substances on the toxin has been reported by Halter,[73] Lippert,[74] and Jungeblut.[75]

Experimental: The present report deals with the effects of gamma radiation from cobalt-60 on the toxic fraction of tetanus toxin and also on the immunizing activity of the toxin following irradiation. A purified toxin (50% pure) furnished through the courtesy of Dr. R. C. Pittenger of the Lilly Research Laboratories, Indianopolis, Indiana, was used for the irradiation study. This toxin was capable of killing guinea pigs in a dilution of 30×10^6 when 1 ml was injected subcutaneously. The toxin was diluted 1:25 with sterile 15% gelatin containing 1:10,000 merthiolate as a preservative. A 0.2-ml portion of the diluted toxin was placed in sterile serological tubes plugged with cotton and the latter irradiated for the periods designated in Table XXXV. Control tubes were maintained at toom temperature throughout the irradiation period of the samples exposed to cobalt-60. After irradiation all tubes were placed in the cold room (2°C) and titrations in mice carried out 24 hours after the original toxin had been diluted for this experiment. The pH of the solution was 7.4 Toxicity for mice was determined by injecting 0.1 ml of the toxin solutions subcutaneously. The results of this study are given in Table XXXV.

TABLE XXXV

EFFECTS OF GAMMA RADIATION (COBALT-60) ON TOXICITY OF TETANUS TOXIN

Number of Mice		Hours Irradiated	Radiation Dose (Rep. in air)	Survival Time, Hours	
Control	Irradiated			Control	Irradiated
2	6	1/2	40,000	24;24	24;24;24;24;24;24
2	6	1-1/2	120,000	24;24	All survived
2	6	6	480,000	24;24	All survived
6	6	24	1,920,000	24;24;24;24;24;24	All survived

* This study was carried out in collaboration with Ray DeHaan, graduate student in the Department of Bacteriology of the Medical School.

Results: From the data given in the table it is apparent that irradiation for 1-1/2 (120,000 Rep. in air) hours with cobalt-60 would detoxify the calculated amount of 25,000 MLD's of the tetanus toxin. This may be attributed to the presence of hydrogen peroxide and free radicals produced by gamma radiation. The activity of oxidizing substances has been shown by a number of workers to have detoxifying action on the same system.

Immunizing Activity of the Detoxified Toxin: Guinea pigs were injected with 0.2 ml of the 1-1/2 (120,000 Rep. in air) hour irradiated and detoxified toxin. This amount of material before detoxification contained approximately 30,000 MLD's for guinea pigs. The animals received 3 injections of the treated toxin for a period of 3 weeks. They were then allowed to rest for 1 week before being challenged with 100 MLD's of untreated toxin. Of the 4 animals used in this study, all died at approximately 72 hours, which also was the time of death for the controls. It is apparent from this preliminary study, therefore, that accompanying detoxification of tetanus toxin, under the conditions of this experiment, there is also a loss in immunizing or protecting activity of the toxin.

w) Experiment No. 23. The Effect of Gamma Radiation (Cobalt-60) on Agglutinating and Precipitating Antibodies.*

Mention has been made in the introductory section of this report of the findings of Brasch and Huber[28] on the relative ineffectiveness of high energy electrons on antibodies to diphtheria toxin in dosages that afforded sterile serums. Thus, it appeared desirable to include the effects of gamma radiations from cobalt-60 on two antibody systems in the present study.

Experimental: Effects of Gamma Radiation on Red Blood Cell Agglutinins. Into each of 5 serological tubes was placed 0.5 ml of serum containing antibodies to mouse red blood cells (this serum had a 1:10,000 concentration of merthiolate as a preservative and was kept at 5°C when not in use). A control tube of the serum was maintained at room temperature while several of the tubes (cotton-plugged) were being irradiated in the cobalt vault. The sera were then stored in the refrigerator for several days, or until tests were made on their agglutinating properties. The sera were diluted with saline solution (0.85% NaCl) in twofold dilutions, following which 0.5 ml of a 2% suspension of mouse red blood cells was added to each tube. The latter were then placed at 37°C for 2 hours and readings of the extent of agglutination in each recorded. These readings were made as (++++) for complete agglutination, (++) for partial agglutination and (-) for no agglutination. The results of this experiment are presented in Table XXXVI.

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* This study was carried out in collaboration with Jack Battisto, graduate student in the Department of Bacteriology.

TABLE XXXVI

THE EFFECT OF GAMMA RADIATION (COBALT-60) ON RED BLOOD CELLS AGGLUTINATING ANTIBODIES

Irradiation Time	Radiation Dose (Rep. in Air)	RBC control (No antiserum)	Agglutinating Titers (Dilution of Antiserum)								
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
0 (unirradiated control)	-	-	++++	++++	++++	++++	++++	++++	++	+	-
1 hr	80,000	-	++++	++++	++++	++++	++++	++++	++	-	-
3 hrs	240,000	-	++++	++++	++++	++++	++++	++++	++	-	-
6 hrs	480,000	-	++++	++++	++++	++++	++++	++++	++	-	-
24 hrs	1,920,000	-	++++	++++	++++	++++	++++	-	-	-	-

Results: From the data given in the table it is evident that exposure of the red blood cell agglutinating serum to cobalt-60 for a period up to 6 hours causes no appreciable effect in the agglutinating activity of the serum (the difference of a (++) reaction in the latter series in the 1:128 dilution and the corresponding value in the 1:256 dilution of the control, unirradiated tube is well within the range of experimental error of this technique). Exposure of the serum for 24 hours (1,920,000 Rep. in air) resulted in an appreciable drop in the titer of the agglutinating serum, from the original 1:256 dilution down to 1:16 concentration.

Experimental: Effects of Gamma Radiation on Type I Pneumococcus Soluble-Specific-Substance (SSS) Precipitins. To each of 5 serological tubes was added 0.5 ml of serum containing antibodies of pneumococcus Type I polysaccharide (SSS). (This serum contained no preservative but was kept at -40°C.) One tube (cotton-plugged) was maintained at room temperature while the remaining tubes were irradiated in the cobalt vault. After irradiation all tubes were stored in the refrigerator for several days, or until determinations were made on the precipitin titers of the various samples. The serum was added to precipitin tubes in an amount of 0.1 ml to each tube. Over the latter was then "layered" an 0.1-ml amount of tenfold dilutions of the homologous Type 1 pneumococcus polysaccharide. The formation of a ring of precipitate at the interface of the two solutions, after 24 hours at 40°C, was read as positive, (+). The results of this experiment are given in Table XXXVII.

TABLE XXXVII

THE EFFECT OF GAMMA RADIATION (COBALT-60) ON TYPE I PNEUMOCOCCUS POLYSACCHARIDE PRECIPITATING ANTISERUM

Irradiation Time	Radiation Dose (Rep. in air)	Serum-Saline control (no antigen)	Antigen Titers (Dilution of Antigen) from 1 mg/ml						
			10^{-0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
0 (unirradiated control)	0	-	+	+	+	+	+	-	-
1 hr	80,000	-	+	+	+	+	+	-	-
3 hrs	240,000	-	+	+	+	+	+	-	-
6 hrs	480,000	-	+	+	+	+	+	-	-
24 hrs	1,920,000	-	-	-	-	-	-	-	-

Results: From the data given in the table it may be noted that the precipitating antibodies used in this study will withstand at least 480,000 rep in air irradiation of gamma rays emitted from cobalt-60. However, the same antiserum exposed to 1,920,000 rep of the rays resulted in a complete loss antibody activity, thus, indicating this system to be somewhat more sensitive to gamma radiation than are the red blood cell agglutinating antibodies studied in the foregoing experiment (page 57).

x) Experiment No. 24. The Effect of Gamma Radiation (Cobalt-60) on the Potency of Several Antibiotics.

Brasch and Huber[28] demonstrated that various drugs could be effectively sterilized by electrostatic impluses from the capacitron with no detectable harmful effects on the chemical or biological activity of the materials. Brownell,[76] moreover, reported on the results of a series of collaborative studies carried out with Parke, Davis and Company in which gamma radiations from the present cobalt-60 source (1000-C) also effectively sterilized several pharmaceutical products. The latter items selected covered a wide range of molecular weights and included some which are heat-sensitive and give some difficulty in sterilization by other methods. In the study, the samples were contaminated with B. subtilis and then irradiated in glass-sealed or rubber-capped containers. The results indicated that gamma radiation from cobalt-60 caused complete sterilization in all instances when exposed for 24 hours (1,920,000 Rep. in air). Calcium gluconate and ascorbic acid appeared to be unaffected by this amount of exposure insofar as the chemical nature of the compounds were concerned. There was about 20 per cent loss in activity in the irradiated hormone "theelin" and almost complete loss

in activity of the hormone "pitocin". Antitoxins for tetanus and a mixture of tetanus-diphtheria toxin with pertussis vaccine appeared to be uneffected as the result of 24hours irradiation insofar as their biological activities were concerned.

Experimental: In the present study several antibiotics were irradiated in the dry state for 24 hours (1,920,000 Rep. in air) with gamma radiation from the same source used by Brownell and the Parke, Davis group. Following irradiation, the powders (along with nonirradiated control samples) were dissolved in sterile distilled water in appropriate concentrations and cylinder assay tests carried out for potency of the various antibiotics, using Staphylococcus aureus as the test organism.

Tests for potency of the treated, as well as the untreated control solutions were carried out 3 days after irradiation. The solutions were then stored in the refrigerator and additional tests for potencies were made on the same solutions 6 days and again 90 days after irradiation. The results of these tests are presented in Table XXXVIII.

TABLE XXXVIII

THE EFFECTS OF GAMMA RADIATION (COBALT-60)
ON THE POTENCY OF SEVERAL ANTIBIOTICS

Antibiotic*	Potencies - Days After Irradiation (Same Solutions)					
	3 days		6 days		90 days	
	Irrad.	Unirrad.	Irrad.	Unirrad.	Irrad.	Unirrad.
K-Penicilin "G"	37mm**	37mm	30mm	32mm	37mm	37mm
Streptomycin KCl	24mm	24mm	18mm	16mm	13mm	13mm
Auromycin	31mm	31mm	25mm	20mm	23mm	24mm
Chloromycetin	23mm	23mm	14mm	21mm	16mm	16mm
Terramycin	29mm	24mm	17mm	20mm	21mm	21mm

* Physical appearance of the antibiotic powders, or crystals, immediately after removal from the cobalt-60 vault following 24 hour's exposure to the gamma rays:

Penicillin: No color change, nor apparent change in solubility in water.
Streptomycin: Slight change to grey of treated powder no change in solubility.
Aureomycin: No change in color, nor apparent change in solubility.
Chloromycetin: Slight change to grey of treated crystals; no apparent change in solubility.
Terramycin: No change in color; no apparent change in solubility.

** Figures represent zones of inhibition against S. aureus in the cylinder assay test of solutions prepared from treated and untreated powders.

Results: In summarizing the data given in Table XXXVIII it may be noted that, in general and within the limits of experimental error of these preliminary tests, there appear to be no appreciable, overall, differences in potencies between the irradiated and nonirradiated samples of the several antibiotics. With the exception of penicillin, which appeared to retain its potency while in solution for 90 days (both in the irradiated and unirradiated series), the remaining solutions showed some losses in potencies which appeared comparable in degree in the irradiated as well as the nonirradiated control solutions. The footnote under the table indicates the physical appearances of the powders or crystals soon after irradiation. A slight change in color, from white to grey, was noted in the irradiated samples of streptomycin and chloromycetin. However, there did not appear to be any differences in the degree of solubility of these samples as compared to the nonirradiated ones.

SUMMARY

Although several of the studies described in this progress report are preliminary in nature, a brief summary of the findings are presented herewith.

1. Ultra violet irradiation of a medium, essential for growth of Lactobacillus arabinosus, will alter it to the extent that the organisms will not develop or grow in the treated medium.

2. High intensity gamma radiation from a 1000 curie cobalt-60 source has been found effective in destroying *Escherichia coli*, *Lactobacillus arabinosus*, *Proteus vulgaris*, *Mycobacterium tuberculosis* and *Trichomonas foetus* with dosages of the magnitude of approximately 3,000 rep in air.

3. Two spore-forming organisms, *Bacillus subtilis* and a spore-forming organism isolated from a sample of milk (M-S strain) required approximately 2,000,000 rep in air for their complete destruction.

4. The yeast, *Saccharomyces cerevisiae*, was destroyed with approximately 400,000 rep in air, and spore suspensions of two molds *Penicillium notatum* and *Aspergillus niger*, by approximately 300,000 rep in air.

5. Gamma radiation from cobalt-60 was found to inactivate Psittacosis virus and human pneumonitis virus in dosages of less than 240,000 rep in air, whereas 400,000 rep in air were required to inactivate the bacterial virus, Staphylococcus bacteriophage.

6. About 2,000,000 rep in air are required to completely sterilize the bacterial flora in raw milk with gamma radiation from cobalt-60.

7. Low doses of gamma radiation from cobalt-60 were found to destroy the toxic, as well as, the antigenic properties of tetanus toxin.

8. Red blood cell agglutinating antibodies were found to be more resistant than pneumococcus carbohydrate precipitating antibodies to gamma radiation from cobalt-60.

9. Dilute solutions of the enzymes, trypsin, papain and pepsin are susceptible to gamma radiations from cobalt-60. High concentrations of the same enzyme systems are, on the otherhand, unaffected by relatively large doses of the radiation.

10. Cystine and pyruvate were found to protect dilute solutions of the enzyme systems from radiation damage from cobalt-60.

11. The phosphatase enzyme normally present in milk was only altered slightly by 2,000,000 rep in air.

12. No appreciable alteration in potency of the dry antibiotics, penicillin, streptomycin, aureomycin, terramycin and chloromycetin, was noted following exposure of these agents to approximately 2,000,000 rep in air of gamma radiation from cobalt-60.

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