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PROCEEDINGS  
SEVENTH CONTRACTORS' MEETING  
QUARTERMASTER COMPTON  
RADIATION PRESERVATION OF FOODS PROJECT

Chicago, Illinois  
6-8 June 1961

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RESOLIVE

QUARTERMASTER COMPTON


QUARTERMASTER FOOD AND LOGISTICS DIVISION  
QUARTERMASTER ENGINEERING AND RESEARCH CENTER  
QUARTERMASTER CENTER FOR FOOD RESEARCH  
CHICAGO, ILLINOIS

Proceedings

Seventh Contractors' Meeting  
Radiation Preservation of Foods Project  
(7-84-01-002)

Presented herein is a summary of proceedings at the Seventh Meeting of Contractors of the Quartermaster Corps Radiation Preservation of Foods Project at Chicago, Illinois 6-8 June 1961. Appreciation is expressed to all those persons who served as roundtable discussion leaders and as secretaries for the technical sessions for their efforts in providing the summaries which have made prompt preparation of this report possible. Similarly, the expeditious submission of summaries of the speeches contained herein has facilitated the preparation of these proceedings by the Quartermaster Food and Container Institute for the Armed Forces.

Approval for release to qualified  
personnel participating in the  
program:

  
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Chicago, Illinois

July 1961

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## INTRODUCTION

The first full-scale meeting in two and-a-half years, of contractors in the Quartermaster Radiation Preservation of Foods Project, was held in Chicago 6-8 June 1961.

The program's objectives were revised in the intervening time, to reflect increased effort devoted to basic research. In addition, the contractors working in specific tasks met in "working meetings" during 1959-60. As a result of these meetings, a large accumulation of specific results and developments needed to be brought before the entire group.

The hope was expressed that this meeting, like its predecessors, would afford a special opportunity for attendees to learn first-hand about other areas of research so that each could assess his own role in the over-all program.

This aim was accomplished, and in addition, rapid dissemination of information was achieved and new ideas were generated.

II. THE QUARTERMASTER RADIATION PRESERVATION OF FOODS PROJECT

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## RADIATION PRESERVATION OF FOOD, IN PERSPECTIVE

Ferdinand P. Mehrlich  
Acting Director - QM Radiation Preservation of Foods Project

Introduction: Ladies and Gentlemen - This is the Seventh Meeting of the contractors of the Quartermaster Radiation Preservation of Foods Project, the first having been held in Chicago at the QM Food & Container Institute for the Armed Forces. The last previous meeting was held some two and a half years ago during a terrific snow storm at the Hotel Moraine-on-the Lake, Highland Park, Illinois. Since that time a number of important developments have transpired, and it seemed desirable to bring together the present group to review the purposes of the undertaking, the progress made and to point up the challenges of the future.

Since our last meeting much of the long-term feeding recommended and initiated by The Surgeon General and his contractors has terminated and a considerable part of the histopathology relating to these feeding tests has been completed.

A re-orientation of the program has been effected and Congress has approved, in principle, the continuation of a substantial effort for a six-year period--to provide military subsistence items preserved by radiation.

Based largely upon the promising indications of the Quartermaster's program, but buttressed by the investigations of a host of others, the AEC has again entered the field with a program oriented towards commercial exploitation of fish and fruit products, the shelf life of which will have been significantly extended through low-dose radiation.

The objective of our seventh meeting is 1) to set forth in perspective the present activities; 2) to define the objectives now to be attained; 3) to differentiate between the areas of primary responsibility of the participating agencies; and 4) to use this background as a base from which hopefully to plot the most efficient attainment of satisfactory military end-items.

This meeting thus is to be a timely stock-taking, re-evaluation and planning period. It is a pleasure to be able to participate in a significant way in this important undertaking.

I will endeavor to chart briefly the initiation and early development of the Project through terse reference to published material. I will confine my report to those historical events giving perspective and meaning to our current activities. Dr. Josephson, who will follow immediately after this speaker, will give details of the current program; the areas of its emphasis; and the kinds of products anticipated to stem from its undertaking. Later, Major Pomerantz will discuss the facilities and equipment to be available in the immediate future for the expedition of our renewed efforts.

Historical:

"Period of low government interest- The general possibilities of the treatment of foods with ionizing radiations were well established on a theoretical and laboratory basis before 1949. Several points were clear at that time. Foods could be preserved with ionizing radiations without more than several degrees rise in temperature. In some cases, however, undesirable changes in odors and flavors, as well as texture and color, were experienced at the sterilizing dose level.

"It had become apparent that the necessary experimentation to obtain the solution to the food technology aspects, as well as the clearance for wholesomeness, would entail time-consuming and expensive programs. The research investment necessary to push the process over the technological hump appeared sizable. Such a risk appeared unreasonably high for industry as a whole to assume, although a few organizations, notably Swift and Company, were subsidizing a significant effort. Universities did not possess sufficient financial resources to go forward on their own. Consequently, it was apparent that the Government was the logical agency to sponsor the program during the early period of uncertainty.

"Early government support- Before 1950 there was only meager Governmental support in research on the irradiation of food. Various agencies had been supporting fundamental research contracts in the nuclear and biological sciences. This was especially true of the Atomic Energy Commission and the Office of Naval Research. However, little thought was given to the food project save for token contractual support in the early 1940's. An appeal by Electronized Chemical Corporation for fiscal assistance from the Department of Agriculture and the Army Quartermaster Corps as late as 1951 was unsuccessful. At that time it was the agencies' contention that private enterprise should be the appropriate body to prosecute research necessary for the development of a commercial process.

"The Atomic Energy Commission Program- During early 1950 the Atomic Energy Commission began seriously looking for ways and means of utilizing the waste fission products which were beginning to accumulate in the nuclear reactor operations. Their potential use in the ionizing treatment of food looked promising. Under the general supervision of the Atomic

Energy Commission, a series of research contracts were negotiated to investigate the possibilities.

"Growth of Army investment- For several years Mr. J. N. Davis, then Special Assistant for Research and Development to the Undersecretary of the Army, Mr. Earl Johnson, urged a concerted effort over a broad front to develop the ionizing processing of food on a practical basis. Inasmuch as the Army Quartermaster Corps was responsible for subsistence research and development for the Defense Department, the Corps reiterated its previous position that the development of manufacturing and processing techniques should be left to private industry. Mr. Davis argued, however, that the military as well as the civilian benefits were so great that the military agencies could not afford to let progress on the project stagnate.

"The Army Quartermaster Corps Program- In August, 1953, the Army General Staff approved the Quartermaster Corps' plans with an allotment of \$660,000 for the first year, and a coordinating staff was established including personnel in the Office of The Quartermaster General, Quartermaster Food and Staleness Institute in Chicago, Army General Staff, and Department of Defense.

"To coordinate the important phase on wholesomeness and nutrition between the Quartermaster Corps and the Medical Corps, an Army Task Group was organized in January 1954.

"At the same time, an Advisory Committee was organized by the National Academy of Sciences to provide scientific advice on the Quartermaster program.

"To obtain further assistance from industry, early in 1956, a Quartermaster Industry Advisory Committee on Radiation Preservation of Food was

organized and held its first meeting 27 March 1956 at the Quartermaster Food and Container Institute.

"The Office of The Surgeon General also formed an Advisory Committee to advise on research into the wholesomeness of irradiated foods, the wholesomeness program being directed from the Office of The Surgeon General. Dr. A. J. Lehman of the Food and Drug Administration provided continued advice on testing protocol and requirements.

"Beginning with 1954, the entire Governmental program on the treatment of food with ionizing radiations, except for a small amount of direct effort by the Atomic Energy Commission and the Department of Agriculture, was borne by the Quartermaster Corps. However, the Department of Health, Education and Welfare has served in a consultative and advisory capacity through its Food and Drug Administration. The Department of Interior has cooperated in preliminary screening experiments on the effect of ionizing radiation on selected fish products. The Department of Commerce has reported frequently on the work to interested trade associations, industry groups, and the general public.

"National Program on Food Irradiation- General Hastings submitted a proposal to the Army General Staff on 7 July 1955 to place the program on a national basis. A fuller participation by all Government agencies was suggested, as well as a five-fold increase of the effort over the next three years. An over-all National Advisory Committee was proposed to guide the Quartermaster Corps in policy matters and the transition of the militarily-oriented program to a nationally-oriented, Government-sponsored program and subsequently into the free economy. The committee would also

interest industry in carrying on further development of the program at the earliest practicable time.

"The National plan was approved by the Secretary of the Army, Wilber M. Brucker, and forwarded to the Secretary of Defense, Charles E. Wilson, on 19 August 1955.

"On 22 November 1955, Department of Defense representatives appeared before the Atomic Energy Commissioners to launch the national program. The briefing was conducted by Deputy Assistant Secretary of Defense for Research and Development, J. B. Macauley, Brigadier General J. P. Daley, Colonel Jackson, Lt. Colonel Cook, and Dr. Siu.

"The current state of the art was explained as well as the basis for the Defense Department's optimism over the eventual success of the program. The need for a more broadly based effort was brought out, with particular reference to the Commission's assistance in the designing, funding, and constructing of a gamma-producing reactor for the irradiation of food.

"The conception of the world's first ionizing processing pilot plant marked the official transition from a purely military-sponsored program to the broader based national program.

"The White House lent encouragement to the project. In October 1955, Dr. J. C. Woodhouse brought the significance of the project to the personal attention of Mr. Sherman Adams, The Assistant to the President. The need for even greater emphasis was expressed, as well as for a nationally coordinated program at the highest level. A meeting was called by Mr. F. Seaton, Administrative Assistant to the President, to consider the matter on 2 November 1955. Present at the White House

meeting with Mr. Seaton were Dr. J. C. Woodhouse, the Assistant Secretary of the Army, Frank Higgins, Major General Hastings, and Dr. Siu. The Army was commended for its past work and the need for more direct and intensive collaboration from other agencies was reiterated.

"To guide the broad national program, on April 4, 1956 it was decided that an Interdepartmental Committee of interested Government agencies would be established (in lieu of a National Advisory Committee, which had been recommended earlier). Initially, the Secretary of the Army was to appoint the Chairman of the Committee but eventually the chairmanship was to go to the Department having primary interest in adapting future development to use by the civilian economy. The functions of the Committee were (a) to provide motivation and broad guidance for the program, (b) achieve a high level of participation by other Government agencies and industry, (c) effect a transition of program responsibilities as rapidly as possible. On 4 May 1956, Secretary Brucker invited the heads of interested agencies to designate their respective representatives. The first meeting of the Committee was held on 15 May 1956 with Dr. William H. Martin, The Army's Director of Research and Development, as Chairman.

"Start of design work for construction of the U. S. Army Ionizing Radiation Center- When design work on the radiation center was started in 1957, it appeared that fundamental problems in radiation preservation would be solved before the facility was due to be completed. The plant planning was started at that time to make possible a quick transition from laboratory studies to pilot plant studies.

"Quick transition was deemed necessary to reduce "lead time" - the time that elapses between the start of a development project and its final completion.

"The Center was to consist of three major components: a gamma source, an electron source, and a food preparation facility.

"On 22 October 1959, the Department of the Army deferred plans for construction of the U. S. Army Ionizing Radiation Center at the Sharpe General Depot, Stockton, California, pending further laboratory studies on the use of high dose radiation as a means of sterilizing food. A revised research program has been developed by the Quartermaster Corps and will be reported upon by Dr. Edward Josephson of the QMR&E Command. Dr. Josephson will follow your present speaker on this program."

Program of the Atomic Energy Commission - Information accumulated under Quartermaster and other programs has appeared to indicate the possibility of prolonging the shelf-life of fish and fruit products through low level radiation -- in a degree to attract possible commercial interest. Exploitation of these potentials, primarily for the civilian market, has become the focal point of a newly developing AEC program. Thus there has been a separation between essentially civilian market interests and military subsistence desires.

Tersely, the AEC program is to "establish the requirements for bringing low dose radiation processing of foods to the point of technical and marketing feasibility, and to conduct the research necessary to fulfill these requirements." In order to fulfill the objectives, studies are being or will be conducted in food technology, nuclear effects, preservation factors, packaging, and wholesomeness.

"Specific food products have been identified as showing promise of benefit through low dose radiation processing: these include haddock fillets, clam, crab, flounder, shrimp, strawberries, grapes, peaches, tomatoes, and citrus fruits.

"In selecting such products, the AEC applied the following criteria: (a) the product must have a sufficient market value to justify research on the particular items; (b) the product must have shown promise of a favorable response to radiation processing; (c) it must be generally available in sufficient quantities to warrant the construction of radiation facilities in a particular area; (d) the advantages of the radiation processed product must have sufficient impact on the market to produce an economic success."

Research on the radiation processing of haddock fillets and soft-shelled clams has been initiated by MIT and the Dept. of Interior's Fishery Technological Laboratory, at Gloucester as a joint effort. Shelf-life extension-storage studies and flavor acceptability tests are being conducted at MIT while the Gloucester Laboratory experiments are directed toward the food chemistry associated with radiation processing. The Stanford Research Institute undertook a study to survey wholesomeness and safety aspects of irradiated fruits and vegetables. The AEC also obtained the services of a committee of technical experts who have made pertinent comments on the conduct of the program.

Irradiators:

The first research irradiator specifically designed for the AEC food program, constructed at Brookhaven National Laboratory, employs 25,000 curies of Cobalt-60 and is designed to permit experimental work

under widely varied, yet precisely controlled conditions. In addition, the control console and safety factors have been constructed so that the operator need not have extensive technical training in source operation. The gamma flux has been found to be approximately  $2.5 \times 10^6$  rad per hour, and the irradiation chamber will hold a package measuring 14x18x6 inches. It is now being installed at the MIT-USDI Technological Laboratory at Gloucester, Mass. A second food irradiator, to be constructed during the current fiscal year, will be located in California to provide an irradiation source for the proposed fruit research.

RADIATION PRESERVATION OF FOODS - FUTURE PLANS

Edward S. Josephson  
Special Assistant for Food and Food Irradiation  
Quartermaster Research and Engineering Command

Mr. Chairman, fellow participants in the Radiation Preservation of Foods Program: I join with Colonel Littlejohn in welcoming you aboard on this occasion and bring you the best wishes for a fruitful conference from General Merrill Tribe and Dr. Dale Sieling of Headquarters, Quartermaster Research and Engineering Command in Natick. They regret that they are unable to attend this conference.

As Dr. Mehrlich said, I will attempt to cover in the time allotted the objectives of the Army's Radiation Preservation of Foods Program and the present six-year program. It is within this framework that we ask for and get funds to do our work, and therefore, it is only logical that we do with the money what we say we will do when we ask for the money. Referring to the policy under which this program is carried out I quote from the official Army document<sup>1</sup> as follows:

"Inasmuch as non military departments are beginning to be in a position of carrying leadership responsibilities for the National Atoms for Peace Program in radiation processing of food, the Army will effect a smooth transition toward focusing on military requirements. Research will be directed toward the objective of procurement from the civilian economy of the first generation of relatively few food items of

1. Revised Army Program on Food Preservation by Ionizing Energy, Department of the Army, OQMG, Washington, D. C. Approved 11 March 1960, p. 2

important military significance. The developmental lead time will be compressed to the maximum extent feasible consistent with the state of the art and a requirement for the prior orderly resolution of the key unsolved fundamental research problems. The Army will work closely with industry during the research stage with the objective of facilitating the earliest possible transition to process engineering and production. Inasmuch as the Army's activity will continue to serve as the backbone of the Nation's effort in this program area, generous collaboration will be extended to other organizations in the joint advancement of the President's Atoms for Peace Program." That's the end of the quote on official Army policy under which we conduct our efforts.

I would like to refer briefly to the six-year objectives which have been approved up and down the line<sup>2</sup>.

1. "To obtain wholesomeness clearances permitting unrestricted use of foods preserved by ionizing energy."

2. "To conduct the necessary basic research to advance the technology of food radiation processing so that items of military significance and of quality far superior to analogous items available in the combat theater can be procured," and to this end primary emphasis will be placed on high dose treated or sterilized pork, smoked ham, chicken, and beef. Secondary emphasis will be placed on those low dose treated or pasteurized items of special military significance.

3. We will "conduct timely economic analyses."

2. Revised Army Program, II Six Year Objectives, p. 3

4. We will "formulate such items into logistic saving components for use in Army ration systems from those in the support areas to and including individual military feeding in the forward areas." In other words, we intend to introduce these items into a military feeding system whether the man be fed in large groups in rear base areas or whether he be fed in small groups or whether he eats alone up in forward areas of hot combat. This, of course, must fit in to the future organization of the Army which envisages rapid dispersion and high mobility in an atomic era.

5. We will "develop components meeting special requirements of the Navy, Air Force and Marine Corps."

6. We will "develop process engineering data for efficient transition into industrial sponsorship."

7. We will "determine whether the transition pilot-production plant will be built by industry, the AEC, the Army, or another organization and to effect the necessary transfer of relevant know-how." As an aside I can say that the Quartermaster Radiation Laboratory now under construction is a research tool. However, it could, if events warrant, be expanded to a pilot production plant.

8. We will "establish a reservoir of basic information for future improvements of radiation processed foods."

So much for the basic six-year objectives. To meet these objectives we have roughed out plans for the completion of work and procurement of selected items for the military ration system. Now this involves more than just conducting our basic research; it involves development, application engineering and writing of suitable specifications which are

compatible with the state of the art and will insure the procurement of wholesome, nutritious and high quality food components.

Our first special objective is to begin operating this Quartermaster Radiation Laboratory in the summer of 1962. We will proceed to develop the requisite data and introduce into the Army supply system irradiated components as fast as we can.

I would like to dwell a moment on the subject of potatoes because this is a matter of serious concern to me as a private citizen. You may know now that the health officials of the Soviet Union have approved for commercial consumption in the Soviet Union potatoes that have been treated with radiation doses to inhibit sprouting. Mr. Khrushchev, if he should so decide, could claim this as another first for the Soviet Union and frankly I am a bit chagrined that we don't have this item and perhaps several other items cleared for human consumption and available in the supply system right now. The prestige of the United States could stand a little boosting and we have done so much work that it appears to me that we should have been first with this.

The program to meet Army objectives is an ambitious one. However, I believe that if we all put our shoulders to the wheel and work together we can attain the goals we have set; namely, to introduce into the Army supply system rations containing irradiated components as soon as possible. The stakes, spelled s-t-a-k-e-s, are great and the opportunity should not be lost. This program of course is not carried out solely by the Quartermaster Corps; we have the cooperation of the Corps of Engineers, the Army Medical Service, the Army Chemical Corps, the

Navy, the Air Force, the Atomic Energy Commission, Food and Drug Administration, Department of Agriculture, Department of Interior, and the National Research Council with its several committees, both those under the sponsorship of the Quartermaster Corps and those under the sponsorship of the Army Medical Service.

I would now like to turn the rest of my time over to Major Reuben Pomerantz who will brief you on the Quartermaster Radiation Laboratory now under construction at Natick. Major Pomerantz.

THE QUARTERMASTER RADIATION LABORATORY

Major Reuben Pomerantz  
Director, Quartermaster Radiation Laboratory Plans and Construction  
Quartermaster Research and Engineering Command

Three and a half years ago, Colonel Mastran and I (Colonel Mastran was then Commanding Officer of the Quartermaster Radiation Planning Agency and I was his Technical Director) outlined to the Radiation Preservation Project Contractors' Meeting at Gatlinburg, Tennessee, the general floor plan and specifications for the U. S. Army Ionizing Radiation Center, which was to be built in Stockton, California. As you well know, that establishment has since been deferred.

There have been some changes in emphasis and direction in our program in the intervening years, but I'm grateful for the opportunity to tell you once more about plans and specifications for an irradiation facility; this time however, the name is somewhat different. It is called the Quartermaster Radiation Laboratory, because, as Dr. Josephson pointed out, it is in fact a research tool. We no longer have to talk about plans, because three weeks ago today, we held the official groundbreaking ceremony. When I left last week, the excavation was nearly completed, and concrete for the foundations and superstructure will soon be poured. By next summer the entire facility will be completed.

Design Concept: Although this is primarily a food irradiation facility, it is being designed so that it can be expanded into a semi-production

facility by enlarging the food processing laboratory to double or triple its currently designed size. The rear of the building can be readily removed and the floor layout for the food processing area extended significantly.

Consistent with the Laboratory's primary objective of serving as a research tool and to conform with required economy measures, the concept of design is based upon the fact that laboratory support will be furnished the QMRL by existing QMRL Command research facilities. The design will also take into consideration the large number of visitors that is anticipated. Basically, the laboratory includes a pilot plant area for food preparation, a taste-test kitchen, administrative support areas, nuclear-radiation laboratory complex, and the control radiation source area.

The laboratory is a one-story L-shape building with a total area of approximately 21,000 square feet. It is divided into an "uncontrolled (non-radiation) area" of approximately 11,500 square feet, and a "controlled (radiation) area" of 95,000 square feet.

Traffic Control: The food preparation area is the largest single expanse. The flow of materials will be generally from the rear to the east of the building. The food will come in through the loading area for pre-irradiation processing, and, after the final packaging will pass through the health physics control point. The nuclear, dosimetry, and health

physics laboratories are in the "nuclear square" which will also function as a control point. The normal food processing personnel will not go beyond the control point, as only persons who have reason to work with either the accelerator or the cobalt source will be permitted in the controlled (radiation) area.

Any visitors who come in through the lobby will remain in the observation corridor, unless they have specific reason to enter the control area. The observation corridor has glass windows throughout for easy viewing of all operations within the controlled and uncontrolled areas. What is going on within the two radiation cells will also be visible by closed circuit TV.

#### Radiation Sources

Linear Accelerator: The linear accelerator is the Varian 24 MEV-18 KW unit which was originally planned for installation in the USAIRC in Stockton, California. Certain modifications have been made to simplify the unit and to reduce research development and constructional cost. The main components of the Linac are the electron gun, located in the Linac irradiation cells; the power supply, located in the modulator room; and the control console, located in the Linac control room.

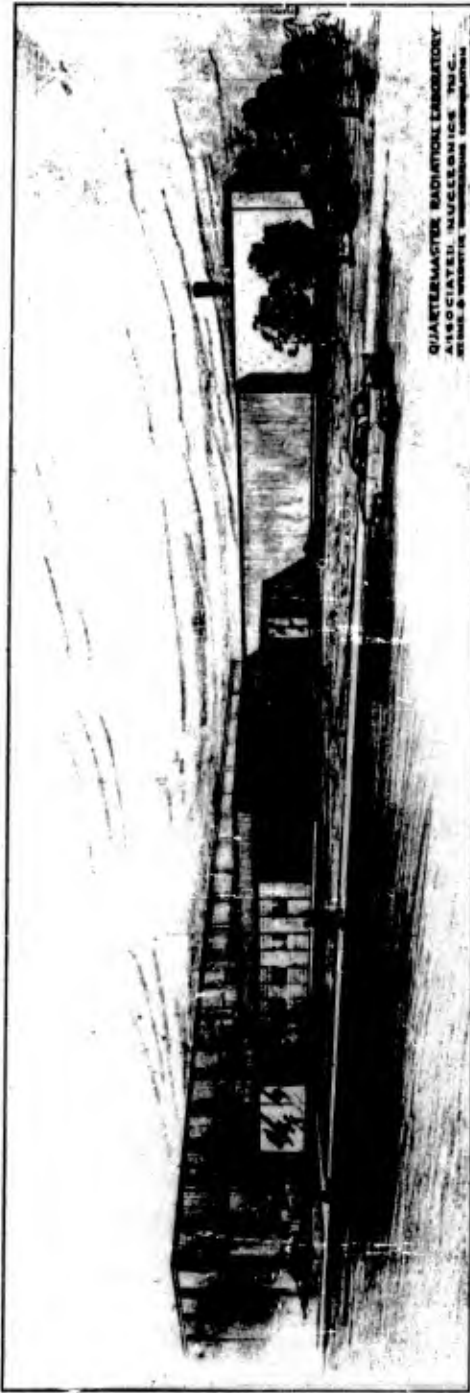
Cobalt-60 Cell: The cobalt-60 gamma radiation complex consists of the irradiation cell with its labyrinth entrance, the source elevator (or storage) pool, the source loading pool and the gamma cell control room.

The gamma cell arrangement generally follows the design developed by the Curtiss-Wright Corp. The cell is designed to contain 3 megacuries of cobalt-60, although the initial loading is only expected to be about 1.38 megacuries. Food packages to be irradiated are carried into the cell by means of a continuous conveyor of the overhead trolley type. To permit entrance to the cell for maintenance or other service work, the cobalt source is lowered to the bottom of the 24' storage pool. A man-trap at the mouth of the labyrinth prevents personnel entry during irradiation while not interfering with the conveyor operation. The interior of the cell may be observed during operation by a TV camera and an optical viewing device.

Although my office at Natick has been charged with the responsibility of designing and planning this building, the customer is the Radiation Preservation Division within the Quartermaster Food and Container Institute for the Armed Forces. We have done everything we can to incorporate within this building all of the desires of the Food and Container Institute personnel. The Institute submitted to us the entire layout of the food processing area. Although we would have liked to have had, and it certainly would have been the Institute's desire, to have more space devoted to supporting laboratory facilities, limited funds did not permit this. Therefore, this building has been designed with the concept that the supporting microbiology and chemistry, as well as other laboratories, would be made available within the Quartermaster

Research and Engineering Center at Natick. Other than the nuclear laboratory, the only laboratory which has been built into this building is for panel taste-testing, because we do not have a taste-test facility at Natick.

In closing, let me point out that this facility is in no way classified any more than the entire Radiation Preservation of Foods Program. Certainly it is open to all of the people in this room and those contractors with whom they associate. It will be open to industry and to those countries who are working with us and cooperating in this program. We hope that one of the annual contractors' meetings, depending on the timing, could perhaps be held in close proximity to this facility so that you can see what we have. Better than that, we would like to show you the complete operation in full swing. I will be here for the duration of the meeting and if there are any specific questions about the QM Radiation Laboratory, I will be happy to answer them.



QUARTERMASTER RADIATION LABORATORY  
ASSOCIATED NUCLEONICS, INC.  
ATLANTA, GEORGIA

III. STATUS OF OVERSEAS FOOD IRRADIATION PROGRAMS

1. Irradiated Food Research in the United Kingdom  
D.N. Rhodes
2. The O.E.E.C. Program in Food Irradiation  
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## THE BRITISH SITUATION

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Low Temperature Research Station  
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Government sponsored research on the radiation preservation of foods is carried out in Britain at two institutes; the Wantage Research Laboratory of the Isotope Division of the Atomic Energy Research Establishment and the Low Temperature Station of the Agricultural Research Council. The programmes of these two groups are in many respects complementary and are coordinated by close collaboration. It is, therefore, on behalf of all of my colleagues that I thank the organisers of this conference for extending to us an invitation to be present. It is extremely valuable to us to have the opportunity of keeping in touch with the current work on food irradiation in the United States. The information we gather enables us to deploy our own much smaller research efforts more efficiently along those lines most productive in furthering our particular interests. These interests do not necessarily coincide with those of the Army Quartermaster, but, although our ultimate aims may differ, I think we share common ground in the need for basic information with which to solve scientific, technical and economic problems.

In Britain we have no research programme aimed entirely at products designed for military use and in the past few years much of our effort has been concentrated upon studying the feasibility of integrating radiation processing with the more conventional food processing methods already in common use. At the same time we have continued laboratory investigations into the fundamental chemistry and physics of the effects of

irradiation upon the components of foodstuffs and into various aspects of the bacteriological problem, and have instituted some animal feeding studies.

I have selected for discussion a number of topics from current British work which illustrate some of the problems which arise in considering the introduction of irradiation processing into practical use and wish to acknowledge the many workers in both laboratories whose work I am now presenting. I would suggest that it is not unlikely that, as your new programme under the AEC develops you may soon be facing similar problems yourselves. As a first step we have made feasibility studies on selected products which appear, on scientific grounds, to offer reasonable hopes of commercial success.

#### The Suppression of Potato Sprouting

This is a well known process in which irradiation does an excellent job, scientifically speaking. It is completely effective, free of undesirable side-effects and presents no difficulties in application when gamma rays are used. However, it has competitors in the various chemical processes which may be used and are equally effective. Chief among these is fumigation by nonyl alcohol. The relative costs of the various processes are shown in Table 1. Irradiation is by far the most expensive when calculated on the basis of the use of a cobalt 60 facility exclusively for potatoes; that is, it would be in use for three months only out of the twelve. If other uses were found for the facility the cost would obviously come down considerably but it appears unlikely that this could be done. Irradiation also differs from fumigation processes in that it must be applied before the tubers are put into store and hence

Table 1

## Sprout Suppression in Potatoes

Process	Cost Dollars per ton
Refrigeration	.70
Maleic hydrazide	1.50 - 2.00
Tetrachloronitrobenzene	.85 - 2.00
Isopropylphenyl carbamate	.70 - 1.30
Nonyl alcohol	.43 - 1.00
Irradiation 2,000 tons	10.00
30,000 tons	4.50
90,000 tons	3.40

the cost of processing is irrecoverable. With nonyl alcohol the application of the process can be stopped at any time if it appears to be uneconomic in a particular season.

We can see little hope of irradiation being applied in this connection in Britain although certainly it may be of value in countries where chemical methods are prohibited by law.

#### Pest Infestation

There are serious problems of pest infestation in various dry products coming into Britain from abroad. In rice, for example, control measures by fumigation have failed for various reasons, and irradiation after sealing in the retail package is a perfectly satisfactory way of overcoming the problem. It is estimated that the cost of applying 15,000 rads at the packaging point could be about 0.2c/lb., an amount which could easily be borne by a first grade product.

Infestation also occurs in dried fruits, which are imported into England in bulk and then packed for retail distribution. Here the amounts of material are comparatively small and although the costs of processing would be partly balanced by savings in inspection and sorting the process is not economic.

On a vastly greater scale there is the problem of the infestation of cereal grains. Studies of this have been made in Australia, where the problem has already been greatly reduced by improving hygiene in storage facilities, maintaining low moisture contents and using fumigants. Irradiation still offers advantages, however, in that it is completely efficient, automatic in operation, free from human errors in dosage etc., and the penetration is accurately known.

The required throughput rate is extremely high when the grain is in motion, for example when passing from elevator to boat, and is too great for irradiation processing with an isotope source; B-ray machines could be used.

#### Salmonella Infections

After a period of rising concern since the war, the Public Health authorities in Britain and other European countries are now seriously agitated at the continued rise in the number of food poisoning incidents resulting from salmonella infections. Certainly a considerable proportion of these arise from contamination in frozen whole egg, a commodity which is extensively used in our baking industry. Although the bacteria are usually killed during baking, re-infection may occur from contamination of cooked goods during handling in the bakehouse and from the inclusion of fillings and creams which contain egg and have not been heated. All the egg imported from many different countries, and that produced in Britain has been found to carry salmonella infection. It would obviously be desirable to make it a legal requirement that liquid or frozen whole egg sold in Britain must have undergone a pasteurisation process sufficient to ensure the absence of salmonella bacteria.

It has been known for some time that egg can be pasteurised against salmonella by heating. However, the process is technically difficult, since the minimum time/temperature conditions for efficient salmonella inactivation are very close to those causing coagulation of the egg proteins. Hence there is very little latitude in the conditions of processing and great accuracy is essential in the maintenance of the temperature of heating surfaces. A slow precipitation is inevitable,

continual stoppages for cleaning are necessary, and additional steps such as homogenisation and filtration of the melange are also necessary.

Irradiation-pasteurisation on the other hand could be applied to the frozen egg in 28 lb. cans as normally produced and the advantages of simplicity in operation and accuracy in processing conditions are obvious enough. We have studied the egg industry in Britain from the point of view of introducing irradiation as opposed to heat processing and have found many features in its favour. Although the production of frozen egg is seasonal, depending upon the spring flush of shell-eggs, the product passes into frozen storage and is held there for long periods before being sold. There would be, therefore, no difficulty in arranging for an isotope irradiation facility to be processing on a continuous basis throughout the whole twelve months, thus achieving maximum efficiency in operation. On this basis we calculate that irradiation processing is slightly cheaper than the heat process, although the capital outlay is greater. Further, in processing egg already frozen, (that is material already in store, or imported unpasteurised from abroad,) the cost of irradiation is some fifteen times less than the alternative of thawing, heating, repacking and refreezing.

The egg industry in Britain is organised by a Marketing Board, which operates under statutory authority, handling practically the whole of the output of shell eggs. The Board also arranges for the breaking out and freezing of surplus eggs when the supply exceeds the demand, and the frozen egg remains the property of the Marketing Board until it is finally disposed of. This means that the flow of egg to and from the frozen stores and the irradiation plants could be regulated from a

central control.

However, we are faced with two problems. Firstly that of organoleptic acceptability. After extensive laboratory trials we are convinced that the baking quality of egg is unaffected by irradiation; the colour is bleached but this is not of great importance in baking; there is an unpleasant odour in the frozen product after irradiation but this does not carry through to the final cooked cake or sponge. Finally the flavour of sponge cakes is almost unaffected by the use of irradiated egg; the difference in panel marking being no more than 0.3 unit on the nine point scale. However, in tests with commercial bakers, whereas three firms found no difference in flavour, two firms reported the irradiated egg quite unacceptable on the basis of flavour. Of course, the final judgment on flavour must be made by the consumer and to test this we wish to set up large scale consumer-acceptability studies. At this point our second major difficulty arises; this concerns the legality of placing an irradiated food on the market, or, in the first place, of supplying a non-voluntary consumer with an irradiated food, which is the essence of an unbiased consumer trial. In Britain, we must obtain prior clearance or permission from the Ministry of Health in order to forestall any possible repercussion from an aggrieved consumer, and application for such clearance would need to be supported by extensive animal feeding tests such as your Surgeon General's Office has so efficiently organised. Unfortunately, (if my literature search has been thorough), you have made no tests on the wholesomeness of irradiated whole frozen egg and the experiments now set up in England will not be complete for another two years. We cannot,

therefore, proceed further in getting the process accepted by the trade until these tests are completed and the health authorities' clearance has been granted. By that time it may be that legislation requiring pasteurisation will have been passed and producers and foreign exporters will have had no alternative but to install heat pasteurisation plants. In this case the introduction of irradiation pasteurisation will have to be made against an established process working within a legislative framework particularly designed for it.

#### Pasteurisation Processing

In addition to these surveys, we are actively investigating the use of pasteurising doses to extend the storage life of a number of products, both at chill temperatures and above.

Broiler Chicken: A part of the British industry is based upon the sale of a chilled as opposed to a frozen carcass. Irradiation can offer a useful extension of the storage life under these conditions. We have investigated storage at 10° and 15° C., temperatures corresponding to the shelf of a retail store display counter and the larder shelf respectively. After holding the carcasses at 0° C. for 1, 4, 7, or 10 days we find that the life at 10° C. is extended by at least 7 days when irradiated by a dose of 0.75 Mrad and at 15° C. by 3 or 4 days. These effects could benefit both the retailer and the consumer.

Fish: Similar advantages can accrue in the distribution and marketing of fish, at least of certain varieties. The flavour of some fish is notably unaffected by irradiation at quite severe doses. For example, lemon sole is hardly affected even at 1.0 Mrad. Cod and halibut,

white fish of low fat content, are slightly affected at this dose, but probably not sufficiently to affect acceptability by an untrained panel. Smoked salmon and cooked crab can stand a dose of 0.5 Mrad. Fresh salmon, however, is very sensitive; a catastrophic drop in acceptability occurs even at 0.3 Mrad and at 1.0 Mrad the product becomes definitely distasteful.

The storage characteristics of these varieties of fish, pasteurised at doses of 0.1, 0.3, and 1.0 Mrad, are now being evaluated at temperatures of 32° and 40° F.

#### Discussion

Irradiation processing can find a place in the food industry in Britain only in open competition with the established methods. It will need to lead the way to new or improved products or to offer a unique or cheaper process. In the reduction of the salmonella hazard in frozen egg and possibly other products and in the pasteurisation of broilers and fish we feel that irradiation has much to offer and the economics in our situation are favourable. The next step in transferring the process from the laboratory to the development scale is, in these and other applications, the testing of the product in large-scale consumer trials, preferably under commercial conditions, since it is difficult to predict the relationship between evaluation by a limited taste panel and the preferences of the consuming public.

However, the public cannot be involved, except on a voluntary basis, until irradiated food has received clearance from the health authorities, and this depends in turn upon the completion and evaluation of wholesomeness feeding tests on animals. In this field we in England are not

so advanced as yourselves, and we do not expect to have any results from our tests for some time to come. Our most pressing need, at present, for the development of irradiation processing is the establishment of the wholesomeness of the product.

THE OEEC\* FOOD IRRADIATION PROGRAM

Pierre Leveque, Director  
European Information Center for Food Irradiation  
Saclay, France

It is not quite correct to speak of the OEEC Program for Food Irradiation, inasmuch as there is no research budget. However, the OEEC has two possibilities for action; a study group for food irradiation, and the European Information Center for Food Irradiation, of which I am the director.

The study group, although it does not meet at regular intervals, holds a one or two-day meeting approximately every six months. It is composed of one or two experts from each member company, plus representatives from the United States and Canada. The international organizations having an interest (International Atomic Energy Agency, Vienna-Euratom-F.A.O.-W.H.O.) send an observer. As a result, there is direct collaboration between these different international organizations.

The study group has several objectives:

- A. The study of the technical and economic feasibility of the practical applications foreseen by the member countries;
- B. Coordination of the radiation programs of the member countries, and if possible, the outlining of coordinated programs;
- C. The enumeration and development of the member countries' sources of radiation;
- D. The proposal, supported by other interested international

\*Office of European Economic Cooperation

organizations, for a meeting of an expert committee to consider the area of wholesomeness;

E. Liaison with the civilian and military programs in the United States and Canada.

I would like to comment a little about the five objectives of the study group:

1. To avoid spreading our efforts too thin, only two applications have been thoroughly studied in all details; these are the irradiation of potatoes to inhibit sprouting and the irradiation of eggs for the destruction of salmonella. The first problem has been studied by France and the report has been published. The second was studied in England and the report on this should be published very soon.

The conclusions of the report on potato irradiation, which are valid only for France, are as follows:

a) The cost of irradiation is 36.7 NF per ton under these conditions: a pilot plant with the capacity to treat 5 tons of potatoes per hour at 10,000 rad (based on 3000 hours of treatment per year); the source, 120,000 curies of cobalt-60 costing \$1.00 per curie, will be amortised over five years.

b) Annual losses are estimated to be:  $3 \times 10^8$  NF in production  
 $5 \times 10^8$  NF in distribution

Despite the high cost of irradiation, its use can be justified, since the average variation in the cost of potatoes between October and the following March is greater than the cost of irradiation (the average based on the last five years).

c) During certain years the production of new potatoes is so great that there is over-production, (which is the case this year) and this doesn't happen without creating social problems.

2. Coordination of programs is the most delicate objective, since each country has its own aims. In addition, certain countries have special fields of interest: Norway for fish, Denmark for dairy products, and Greece for fruit. Other nations are engaged in extensive programs. One method for reaching this goal is to give to each member country as rapidly as possible the results of the studies undertaken. This was the basis for the creation of the European Information Center which is now located at Saclay in facilities made available by the Institute of Nuclear Science and Techniques. The Center publishes a quarterly newsletter which is widely distributed (4000 copies, 2500 in English, and 1500 in French). There is a correspondent in each member country, two in the United States, one in Canada, and one in each interested international organization. These correspondents send in information and make the distribution of the bulletin in their own countries. At this time, the bulletin is distributed without charge.

3. The census of irradiation sources, both in existence or planned, has been made. Each country has at least a source of 1000 curies. For the most part, these are cobalt-60 sources. Some countries (England, France, Belgium) make use of the spent fuel elements from nuclear reactors. In order to conduct research on a large scale, one must look forward to the installation of two sources of 100,000 curies of cobalt-60. The only one of this size presently in existence is in England (150,000 curies at Wantage) and this is reserved for a year in advance for other

work. The Lyonnais Center for the Application of Atomic Energy (C.L.A.A., Lyon, France) has a cave for 200,000 curies of cobalt-60, but at the present time it is equipped only with 3,000 curies.

4. In France, a committee of experts belonging to all the scientific activities interested in the irradiation of foods has outlined a series of tests which must be successfully passed by any firm desiring to obtain authorization to put on the market a food which has been treated by irradiation. (Information Bulletin Nr. 2.)

5. The relationships with the United States are effective and fruitful. In general, the European programs are closer to civilian interests than to the military. In all cases, the studies undertaken in the United States are of valuable help and encourage us to continue our own experimentation in this area. My own presence at this meeting proves, if that were necessary, the importance we attach to direct contacts between American and European research workers.

IV. PRESENT STATUS OF RADIATION RESEARCH

- |                                    |                |
|------------------------------------|----------------|
| 1. Biochemistry of Sensory Changes | A.S. Henick    |
| 2. Product Development             | F. Heiligen    |
| 3. Radiation Microbiology          | M.D. Schneider |
| 4. Nuclear Effects                 | J.P. Berg, Jr. |
| 5. Container Technology            | G.E. Tripp     |

## CHEMICAL AND SENSORY CHANGES IN IRRADIATED FOODS

A.S. Henick, Chemistry & Microbiology Branch

Irradiation processing of foods was initially proposed as a method of food preservation because of its known ability to inactivate or destroy microorganisms, including those of the spoilage type. It is immediately apparent, therefore, that ionizing radiation is capable of producing chemical changes in the material of organic matter and therefore in the components of foods. What could not be apparent was the amount and nature of these changes, and specifically the desirability of these changes from the standpoint of the producer and consumer of irradiation processed foods. It was necessary, therefore, to undertake an extensive research program in order to study these changes and to evaluate their impact upon the quality of the processed foods. This survey will consider the chemical changes observed and their effects upon the flavor, appearance and texture of some selected, typical food items.

### CHEMICAL CHANGES

Food stuffs are composed of varying proportions of proteins, lipids, and carbohydrates in an aqueous dispersion with smaller, but often important, components including vitamins, minerals, pigments, etc. All of the components are subject to chemical reactions resulting from the impact with ionizing radiation. These may be direct or indirect effects depending upon whether they result from impact upon the substrate or from the ionizations in the wake of the impacting particles.

The reactions of water, the major components of many foodstuffs, are well characterized. For this review it is sufficient to say that all possible ions and free radicals of oxygen and hydrogen alone and in combination, have been reported. These ions and radicals may interact upon each other, producing both inert and reactive compounds, or with other components in foods. In all probability the majority of radiation induced reactions in food components are of the indirect type - that is, are reactions with ions and radicals from the water.

#### Reactions of Proteins

Protein studies have been aimed primarily at determining those irradiation induced reactions which cause changes in the quality of food products with a view toward reducing or counteracting the undesirable effects. A secondary but very important interest is in the acquisition of a better understanding of the damaging effects of ionizing radiation upon protein and nucleoprotein of microorganisms and their relationship to microbial destruction. This study has proceeded in two directions: investigations of the constituent parts of the molecule - the amino acids, and of the intact macromolecule itself. Amino acid studies were undertaken because such systems are simpler and therefore easier to control than those of the complex protein macromolecules.

1. Amino Acids. The principal reactions in the decomposition of amino acids in solution by ionizing radiation are deamination and carbonyl formation at the  $\alpha$  carbon. Products include ammonia, hydrogen, carbon dioxide, formaldehyde, formic acid, the parent fatty acid, the fatty acid of one less carbon, and the amine of one less carbon, as well as

the  $\alpha$ -keto acid. All products are formed when radiation is conducted in the presence or absence of oxygen, but the presence of oxygen suppressed reductive deamination to the parent fatty acid and enhanced oxidative deamination to the acid with one less carbon.

The extent of these reactions when the amino group is bound in peptide linkage is not entirely clear. Deamination of N-terminal amino acids in insulin has been reported. The inner amino acids of peptide chains would not be expected to undergo deamination unless it were preceded by hydrolysis. The possibility of such cleavage has been reported in experiments with simple dipeptides, such as glycyl glycine.

Sulfur containing amino acids, cysteine, cystine and methionine appear to be especially susceptible to the free radicals produced by radiation. Cysteine is oxidized to cystine, cystine is subject to oxidative breakdown with the formation of hydrogen sulfide and other products, among which have been reported the sulfonic acid, cysteic acid. Sulfinic acids would also be expected. Methionine has been implicated as the precursor of methylmercaptan in irradiated meat.

The ring-containing amino acids, phenylalanine, tyrosine, tryptophan, histidine and proline are also subject to profound changes. The first three contain a benzene ring which is subject to hydroxylation. Cleavage of the imidazole ring of histidine has been postulated, and the destruction of proline reported.

Some of the amino acid reactions mentioned above may be important in the flavor changes produced by irradiation. None appear to result in measurable decrease in the content of essential amino acids in

irradiated protein, although considerable losses of amino acids have been reported from several proteins.

Proteins. Irradiation results in denaturation, fragmentation and polymerization in proteins. Denaturation has been measured chiefly by alteration in viscosity or in solubility. Changes in solubility have been observed to continue long after irradiation had ceased. Irradiation effects upon dry proteins are not identical to those on protein solutions. In general, dry proteins fragment, whereas solutions tend to polymerize.

Changes have also been reported in the binding capacities of irradiated protein. Lipoproteins, both natural and synthetic, showed less change during irradiation than did the constituent protein. It was also reported that natural lipoprotein protected other protein from some irradiation induced changes. Binding of labeled methionine is increased by irradiation and has provided an extremely sensitive method for detecting molecular changes in protein and protein complexes. On the other hand, a decrease in the sites capable of binding anionic dyes was reported.

A color change which occurs during the irradiation of meat has been related to oxidative changes in myoglobin. Other oxidations, producing protein peroxides, have also been reported.

Reactions of Lipids. In general, the reactions which occur during the irradiation of fats and fatty acids are oxidation, decarboxylation, hydrogenation and dehydrogenation. The magnitude of these changes is probably too small to detract from the wholesomeness of the foods, but may be important in the acceptance or stability of foods.

Oxidation reactions during and after irradiation are similar to those of thermal autoxidation. They depend upon the antioxidant content, the number of double bonds, the amount of irradiation and its rate of application, the presence of oxygen during or after irradiation, and the length and temperature of storage. An interesting new finding shows that saturated fatty esters, for example methyl myristate, are oxidized to produce compounds similar to those produced by the unsaturated ones. These include carbonyls, reducing compounds and peroxides. In the presence of oxygen the amounts of these compounds, especially the peroxides, are increased. It also appears that antioxidants do not prevent the formation of peroxides.

Destruction of antioxidants in lipid systems is an important accompaniment of irradiation. This leads, of course, to a decrease in stability during subsequent storage.

The relationship of these chemical changes to flavor changes will be considered below.

Reactions of Carbohydrates. Although the carbohydrates are a major constituent of foods, especially those of plant origin, they are not particularly radio-sensitive. These chemical changes which do occur, however, may be important because of flavor or texture changes.

In simple sugars, monosaccharides, in aqueous solution, the primary reaction appears to be oxidation of the hydroxyl groups. The primary alcohol at C<sub>6</sub> is oxidized to produce a uronic acid. Irradiation of most sugar-amino acid systems produced typical browning reaction products, and even greater browning occurred when the dry sugar and amino acid were separately irradiated and combined as 50% solution.

Disaccharides are hydrolyzed by very high doses of ionizing radiation. A browning reaction also occurs from the irradiation of organic acid sucrose mixtures. This browning appears to be caused by some reaction other than that with hydroxymethylfurfuraldehyde, which is the reaction most probable in the heating of organic acid and sugars.

Polysaccharides are altered by irradiation as is evident in the loss of firmness during the irradiation of fruits and vegetables. Cellulose undergoes measurable degradation not only during but after irradiation. A partial explanation for the after effects may be in the production of stable, or at least slow to decay, free radicals. Starch is degraded in a manner similar to that for cellulose. Irradiation of dry starch produces a completely water soluble material. Pectins are also degraded, primarily by the breaking of glycosidic chains. None of these carbohydrate changes appears to affect flavor, but many are related to texture and color changes in foods.

#### Reaction of Other Components

##### 1. Vitamins

a. Water soluble vitamins. Ascorbic acid and thiamine are readily destroyed by irradiation, so much so, in fact that their use has been proposed to prevent other radiation induced quality changes. Riboflavin and niacin, on the other hand, are relatively stable, while pyridoxine appears to be midway between these. The other water soluble vitamins, B<sub>12</sub>, pantothenic acid and folic acid are extremely sensitive.

b. Fat soluble vitamins. Vitamin A and the carotenes are very radiolabile. In some foods, protection appears to be afforded,

especially where the carotenes are protein bound. Vitamin E, ( $\alpha_1$ -tocopherol), is a good antioxidant, and, as might be expected, is destroyed by the oxidative conditions produced by irradiation. Some forms of Vitamin K are extremely sensitive, whilst others, particularly those in alfalfa and spinach, are quite stable.

## 2. Enzymes

Although enzymes are proteins, and although proteins have been shown subject to considerable change by ionizing radiation, enzymes have shown rather considerable resistance to inactivation, at least in foods. Heat inactivation of enzymes is usually complete prior to microbial sterilization, whereas radiation sterilization, in general, is insufficient to achieve enzyme inactivation in most foods studied. The effects of enzymes on processed foods vary with the enzyme system. Exclusion of oxygen after processing can be the equivalent of inactivation of oxidase-type enzyme. In irradiated foods, then, the hydrolytic enzymes assume the greatest importance, and of these those in meat have been most studied.

Relatively little recent work has been done specifically on the radiation reaction of enzymes in meat. Rather, the enzymes themselves have been isolated and characterized. A whole series of cathepsin-like proteases have been found in beef muscle tissue, and a considerable body of information accumulated on their properties, such as pH and substrate specificity. Some of these enzymes can be inactivated by the removal of  $Fe^{++}$  ion. It is hoped that this information will be useful in devising procedures for inactivating or controlling these enzymes while minimizing other changes in the

processed foods.

#### FLAVOR CHANGES

Some of the biochemical changes referred to above may be related to specific flavor and odor changes produced during irradiation processing. However, at the present time, the specific mechanism of flavor change is not well enough understood to treat it as an established chemical reaction. Rather, it is more helpful to consider the flavors produced and to try from them to gain some insights into the chemistry involved.

Nearly all foods appear to undergo some flavor change in processing, and radiation processing is no exception. The desirability of any flavor change depends primarily upon the judge, but most unfamiliar experiences are, at least at first, judged undesirable. Here again radiation changes were no exception. The area of the biggest changes, and probably least desirable per se, is in meats. As the carbohydrate content of these tissues is low, it is more reasonable to look to protein and fat for the origin of these changes. Some preliminary correlations are already possible.

At least two new or off-flavors have been recognized in radiation processed meats. One is widely held to be a unique radiation produced flavor usually described as wet-dog hair. Another, which may be related to it has been called burnt feathers. Flavors typical of fat rancidity have also been reported, and these may or may not contribute to the unique radiation odors.

A considerable body of data has been accumulated on the volatile

compounds associated with irradiation. These have been steam vacuum stripped concurrently and consecutively with irradiation, absorbed on activated molecules, chromatographed, mass spectroscoped, derivatized, and most recently synthesized. Compounds have been identified which contain, along with carbon, hydrogen and oxygen, sulfur, nitrogen, one or both, in all states of redox. In overly brief summary, no one compound or combination of few compounds has yet been correlated with the unique irradiation off-flavor.

At present there appear to be two divergent views on the causation of radiation flavor in meat. One view holds that this flavor results from a specific reaction on a specific precursor. Research directed toward the isolation of the precursor and its radiation product has been underway. A substance which fractionates with the phospholipids of beef has been isolated which appears to contain all of the specific radiation odor. Tentatively this white, waxy material has been described as a cerebroside.

Another view holds that the radiation odor results from more general reactions of the protein of meat. Among these general reactions, oxidative cleavage of disulfide linkages and carbonyl formation have been studied intensively. Mercapto carbonyls related to methional have been isolated and identified in the volatiles collected from irradiated meats, as have sulfones and other sulfur compounds. Studies are underway, not only on the identification of compounds, but also on the decay of free radicals in an attempt to solve this complex problem. In one study, a synthetic approach to irradiation odor is being pursued.

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### UNSOLVED PROBLEMS

After 8 years of intensive study, much has been learned about the chemical changes which accompany radiation processing of foods and food components. Much remains to be learned and to be applied to the solution of specific problems. These problems, for the most part, fall into three categories: (1) unwanted flavor changes, (2) unwanted or uncontrolled texture changes, and (3) unwanted color changes. Meats represent the most important substance in which these unwanted changes occur, but similar problems exist to a greater or lesser degree in all types of foods.

There is still a great deal to be learned about the origin of off-flavor in irradiated beef. That it arises primarily from protein changes, with a contribution from lipid changes is most probable. Whether it is due to a single precursor or to a general reaction of a number of unrelated molecular species, whether it is due to a direct radio chemical effect or to a free radical mechanism, to what extent it is influenced by species, age, and sex of the animal - these are major unanswered questions. The relationship of off-flavor to volatile compounds isolated from irradiated meat is not yet established. When these and similar questions are resolved, practical means of control of this problem is more likely to be forthcoming.

Texture problems are in one sense even more difficult to resolve, since there is no agreement on what is desirable texture or on how to measure it. Is softening of the food tissue due primarily to radiation damage in the components or to residual enzyme action? If the former, how can it be prevented or controlled, and if the latter, how



## TECHNOLOGY OF IRRADIATED FOODS

Fred Heiligman  
Food Radiation Technology Branch

At the inception of the Quartermaster Food Radiation Program there were many potential food preservation applications of ionizing radiation. It was known that irradiation could be used to inhibit sprouting in root crops such as onions and potatoes; to deinfest grains and spices; and to destroy parasitic organisms such as trichina. Sub-sterilization doses also aided in controlling food spoilage microorganisms, and higher doses sterilized foods packaged in hermetically sealed containers.

It was early noted that the radiation treatment, while accomplishing its intended effect, in many instances caused certain adverse changes. Potato tubers became more susceptible to storage rot when radiation was used as a sprout inhibitor; it seemed loaf volume was affected when radiation deinfested flour was used for baking, and unusual odors and flavors and changes in texture and color were often produced when radiation was used to control spoilage microorganisms. The degree of these adverse effects were directly related to the dose delivered to the food.

Through continued research, a great deal of information has been generated concerning the character, causes, and effects of some of these radiation induced changes as well as the development of methods and techniques for their control. Much of the information reported here has resulted from investigations conducted by various agencies in cooperation with the Radiation Division of the QM Food and Container Institute.

The application of radiation in food preservation has been placed into two broad categories based on dose: low dose irradiation involving

doses up to 1.0 Mrad and high dose (or radiation sterilization) involving doses above 1.0 Mrad.

In the low dose area, efforts have been concentrated on the application of radiation for sprout inhibition, grain deinfestation, and pasteurization of perishable commodities.

Irradiation doses in the range of 7,500 to 10,000 rad are ample for commercial sprout inhibition. In Russett Burbank, Katahdin and other good storage varieties, storage rot seems to be no problem when tubers are treated at this level. The time of treatment, in relation to harvest time, is not too important providing the tubers are given an opportunity to "cure" beforehand and are treated before breaking dormancy. Dehydrated potato products, made from irradiated tubers, have been rated acceptable but slightly inferior to non-irradiated controls. A dose of 12,000 rad completely inhibits rooting and external sprouting and greatly reduces the occurrence of internal sprouting in onions without detrimental effects. Radiation in the range of 25,000 to 50,000 rad will destroy insect eggs, prevent the development of succeeding metamorphic stages, and will impede reproduction in the adults. Live insects will survive the treatment; however, their life span will be curtailed. Doses of 75,000 to 100,000 rad are required to kill the adults.

Studies on the application of low dose irradiation for extension of the market life of fresh products, a process commonly referred to as radiation pasteurization, have shown the process has a great potential. Dose levels required to control many microorganisms involved in spoilage of fresh products have only a slight effect on sensory characteristics. With microbiological spoilage of this type under control, the product's storage

stability is greatly increased.

Irradiated fruits and vegetables are generally characterized by softening, loss of distinctive bouquet, and some discoloration during storage. Some of these adverse changes seem to progress during continued storage. Softening has been related to radiation induced changes in the structural components of the tissue, namely pectins and cellulose. However, the refrigerated shelf life of many fruits and vegetables can be materially extended with satisfactory retention of quality attributes.

Of particular interest are the results that have been obtained in the radiation pasteurization of meats and seafoods. Doses of 500,000 rad have been shown to increase the refrigerated shelf life of raw beef steaks and raw and enzyme inactivated shrimp several-fold with only a slight effect on the preference scores of the products. These irradiated products have been found acceptable after six months storage; without treatment they would have spoiled in a week or two. The following tables illustrate the results that have been obtained.

Table I

Mean Hedonic Ratings for Raw Beef Steak		
Storage (Weeks)	Non-Irradiated Frozen Control	Irradiated* 38° F. Storage
1	7.0	6.8
4	6.1	7.1
10	7.2	6.8
21	7.2	6.6
27	6.6	6.7

\*500,000 rad

The results of preference tests on irradiated samples and non-irradiated frozen controls are shown above (Table I). These products were scored on the nine-point hedonic scale with "dislike extremely" scored as one, "neither like nor dislike" scored as five, and "like extremely" scored as nine. The steaks were U. S. Good rounds, packed raw in cans under vacuum. The controls were immediately quick frozen at  $-20^{\circ}$  F. and held at this temperature until evaluated. After treatment with 500,000 rad, the irradiated steaks were stored at  $38^{\circ}$  F. There is little or no difference in the scores received by the two lots, even after six months' storage.

Results of similar studies on both raw and enzyme inactivated shrimp also show that 500,000 rad will materially increase their refrigerated shelf life (Table II).

Table II

Mean Preference Scores for Raw Shrimp		
Days Storage	Non-Irradiated Frozen Controls	Irradiated* $38^{\circ}$ F. Storage
14	7.2	7.0
35	6.9	6.7
82	7.5	6.9
104	7.1	6.4
149	7.2	6.2

\*500,000 rad

The shrimp were handled in the same manner as the steaks. Although the frozen controls rated higher than the irradiated samples, both products were in the acceptable range. Enzyme inactivated shrimp also

responds well to the pasteurization treatment.

The uses of sub-sterilization radiation represent only a small part of the Food Radiation Program. Greater emphasis has been placed on the development of sterilized products which will be stable for the long periods of time and under the varied conditions of temperature and humidity which might be encountered in military distribution and storage of foods.

Much of the early work centered on radiation of raw foods; stability required, however, that the autolytic enzymes present be controlled or inactivated. Although free from spoilage microorganisms, the products rapidly deteriorated, developing off-odors, excessive amounts of drip, bitter flavors, and other evidences of enzyme changes.

Enzyme resistance to the irradiation doses used for sterilization has imposed requirements for enzyme inactivation by other means. Since heat is the only effective agent known, numerous methods for heating, including standard cooking methods, infra-red and micro-wave heating, have been studied to develop methods for enzyme inactivation and to determine the effects of heat on the products.

It is clear that the internal temperature is the most important factor; thus, when adequate internal temperatures to control enzymes are attained, the resultant products are partially to fully cooked. For meats, an internal temperature of 160° F. can be considered as minimum within practical periods of time. Increases in certain nitrogenous compounds during storage indicated incomplete enzyme inactivation at internal temperatures less than 160° F. Environmental temperatures used for enzyme inactivation are reported to have little or no effect on preferences in beef;

however, pork chops inactivated in a steam retort at 10° F. above the desired internal temperature were preferred to those heated to the same internal temperature in steam at 212° F.

Texture:

Radiation sterilized meats are often described as being "mushy," "livery," "gummy," "dry" or "friable." Studies of the combined effects of enzyme inactivation and radiation on texture degradation have shown that both contribute to the problem since both cause degradation of beef muscle fiber proteins. Unlike irradiation induced flavors (to be discussed more fully later) which seem to decrease during storage, radiation induced textural changes apparently continue during storage. The decline in preference scores of irradiated meats held in extended storage, after they have reached a peak, seems to be associated with continued texture deterioration rather than flavor.

Humidity as well as heating temperature affects the time required for enzyme inactivation; at high humidities the desired internal temperature is reached in much less time than at low humidities, temperature being held constant. It is reported that a time/temperature relationship (at a constant relative humidity) exists in the degradation of meat tissue constituents. The presence of hydroxyproline (a normal constituent of collagen and elastin) in water, and in calcium chloride extracts, suggests that some of this degradation may be associated with the destruction of connective tissue and collagen, which upon prolonged heating in the presence of water will yield gelatin. Analysis for hydroxyproline during various stages of processing show that radiation at sterilizing doses is more destructive to connective tissue than heat enzyme

inactivation. Radiation changes in irradiated beef products are also manifested in changes in water holding capacity, swelling, pH, irradiation flavor and the amount of extractable connective tissue.

Storage appears to promote a process of reconstitution of some of the degradation products. It has been reported that after four months' storage of irradiated beef, a reduction of free water with a corresponding gain in protein bound water occurred, swelling decreased, and less irradiation flavor was evident. Storage had little or no effect upon water soluble and trichloroacetic acid soluble nitrogen. It is reported that during storage, the texture of roasts made from cutter/canner grade beef improves; whereas, in roasts made from choice grade beef, there is additional deterioration.

#### Appearance and Color:

The chemistry of pigment changes in irradiated meats is fairly well understood. The pink discoloration associated with cooked radiation sterilized meats is due to reactions involving the hemoglobin component in meat. Irradiation causes a reduction of hemichrome, which is brown, to hemochrome, which is pink. Although this discoloration rapidly reverts to the normal color of cooked meat on exposure to air, its significance to product quality requires further investigation. It is interesting to note that this pink discoloration seems to be more pronounced in beef than in pork or chicken; and in chicken, it is more noticeable in the dark meat. The pink color of irradiated chicken develops during storage and not during irradiation.

Chicken samples stored in air at 100° F. for three months developed little off-color whereas those stored in a nitrogen environment became

objectionably pink. The degree of heat treatment applied during enzyme inactivation influences the development of the pink discoloration during storage; samples heated to internal temperatures of 185° F. discolored less during storage than those heated to 155° F. Microwave heating also appears effective in reducing the abnormal pink color in chicken.

A few experiments have been concerned with product appearance. In one, radiation sterilized roast pork and non-irradiated controls were scored similarly for appearance over nine months of storage. In pre-irradiated fried foods, particularly breaded items, a bleaching of the breading occurred which distracted from the eye appeal of the products. When irradiated products are breaded and fried prior to serving, the color and appearance are normal.

To my knowledge, no formal studies have been conducted delving into the effect of appearance of the products in their containers on later acceptability. Usually, the evaluating panelist does not know how a product looks in its container: As we all know, thermally canned foods usually have an excess of fluids, and in meats, the congealed fats make a very unappetizing appearance.

#### Odor:

As previously mentioned, irradiation induces off-odors which are sometimes quite offensive. Since these compounds usually concentrate in the head space gases, inclusion of activated charcoal effectively reduces their intensity. Other agents such as silica-gel and oxygen scavengers, appear less effective.

The intensity of irradiation odor, as well as irradiation flavor, does not seem to follow any particular pattern and cannot be predicted. It has

been noted frequently, that a specific product will differ tremendously in irradiation odor intensity from a similar product produced, as far as we know, in exactly the same way. The only explanation we can suggest for this lack of reproducibility lies in the uncontrollable variations in raw products.

Flavor:

The problem of off-flavors in irradiated food is probably the most serious of all to the food technologist. The effects of variations in raw materials, enzyme inactivation, additives, packaging environments, culinary techniques and other factors on irradiation flavor have been rather extensively studied and some techniques have been developed which aid in reducing off-flavor.

One fact has been fairly well established; irradiation flavor decreases during continued storage until it reaches a certain level and then remains constant. Rate of decrease appears to be related to the storage temperature. Products improved more rapidly at the higher temperatures.

Inclusion of activated charcoal packets in containers improved preference scores of both pork and chicken, particularly after storage for relatively long periods of time. No benefits have been assessed, as yet, to the use of charcoal in beef. It is possible that the chemical entities involved in irradiation flavor in beef are different and are not absorbed, or, they may be present in greater quantities in beef than in the other meats studied, and not enough charcoal has been added to be effective.

Although high vacuum packaging is advantageous with respect to sensory qualities in irradiated products, the efficacy of oxygen scavengers, such as glucose-oxidase, is questionable. In experiments studying the effects of

oxygen scavengers, adequate controls to ascertain that the scavengers were functioning in the removal of residual oxygen as planned, were not included; thus, the results may not be valid. Oxygen appears to play an important role in the development of off-flavors in irradiated meats. Measurable improvement was attained through reducing pre-irradiation exposure of raw beef to oxygen.

The intensity of irradiation flavor is also influenced by the thermal state of the product during irradiation. This effect is not very obvious at temperatures above freezing; however, at temperatures below freezing, the degree of radiation induced changes is apparently markedly reduced. Products irradiated in liquid nitrogen ( $-312^{\circ}$  F.), for example, show a minimum of radiation induced changes. We can only speculate as to the mechanisms involved; it could involve immobilization of free radicals, decrease in availability of oxygen through liquefaction at the very low temperature, or perhaps reduction of water-involved reactions. Irradiation at acetone-dry ice temperature ( $-103^{\circ}$  F.) results in some flavor improvement, but the effect is not as dramatic.

Attempts to suppress irradiation flavor through the use of additives have usually resulted in limited success. Some improvements have been obtained through the addition of MSG, liquid smoke and charcoal salt. In beef patties, sodium nitrite, ascorbic acid, salt, pepper, and tomato paste were effective in reducing irradiation flavor intensity. The products received poor preference scores probably because the patties looked like hamburgers but had strong and unnatural flavors and colors. There are some indications that a tomato component may inhibit or reduce radiation induced off-flavors. It has frequently been noted that when tomato

is added, either prior to or after irradiation, the products received acceptable preference scores. The mode of action of tomato as it influences flavor is not known, but is being studied.

Variations in the raw product also influence irradiation flavor intensity. For example, one type of muscle will often have less irradiation flavor than another even though both were treated similarly; working muscles from the rounds are frequently lower in off-flavor than non-working muscles in the loins.

#### Culinary Practices:

Special culinary techniques are playing an important role in producing acceptable products. Generally, the poor acceptance accorded various irradiated beef items has been attributable to noticeable irradiation flavor and texture deterioration. However, a number of beef items made by special culinary treatments have received good preference scores; these include Italian beef, stew and pot roast. Ready-to-eat Spanish steak and meat loaf, both of which can be heated in their containers, have been developed.

Roast pork made from loins, fresh hams, and Boston butts, enzyme inactivated in steam and in dry heat, have been compared. Those made from the loins and fresh hams were well received. Items cooked in steam appear to be preferred probably because they seem more moist. Irradiated pork has been used to prepare many well accepted items such as pork and noodles, stuffed pork chops, pork chop suey, and grilled pork chops.

The use of such additives as ascorbic acid, nitrite, vegetable extracts, by "injecto" pumping, have not improved the quality of roast pork. However, the inclusion of barbecue sauce in the pumping solution did result

an substantial improvement. The barbecued pork pictured earlier received excellent preference scores on initial evaluations.

The adverse odor, flavor, and color occurring in radiation sterilized chicken can be significantly reduced by appropriate culinary procedures. Odor and flavor changes can be reduced by appropriate cooking such as deep fat frying. Barbecuing is another method which appears to reduce irradiation changes. As was the case with beef and pork, irradiated chicken has been the basis for many well accepted items such as chicken a la king, chicken salad, chicken cacciatore and fried chicken. Irradiated ham items, prepared from a large variety of commercial and specially cured pork products have been evaluated; those prepared from boneless smoked ham and boneless smoked butts were considered best. Nitrate, phosphate, ascorbate, and dextrose, added to a nitrite salt pickling solution, did not improve preference scores.

It has been noted that irradiation has an effect on cured meat color, interfering with the conversion of total pigment to cured meat pigment. During storage, however, there is some reversion to cured meat pigment.

Many waterfoods have been well received shortly after irradiation, but in storage, they darken and develop adverse texture changes. Packaging in high vacuum is beneficial in retarding these changes in shrimp; however, this alone is inadequate to give the product the required stability.

Considerable difficulty has been encountered in breaded deep fat fried items. Irradiation causes bleaching and gummy texture in the bread-  
ing. A corn flour based breading has been developed which seems to resist changes during irradiation and therefore may prove to be adaptable.

Storage Stability:

Frequently, radiation sterilized meats which have received no treatment other than enzyme inactivation are not well accepted because of unusual color, odor, texture and flavor. However, several irradiated meats that were enzyme inactivated by cooking have received good scores (Table III).

Table III

Irradiated 4.5 Mrad	Mean Preference Scores for Irradiated Meat Items	
	Storage Temperature 70° F.	100° F.
Pork Chops	7.5	6.4
Pork Loin	7.2	6.2
Chicken Parts	7.2	7.2
Beef Steak	6.9	6.2
<hr/> 25-month storage		

The mean preference scores of one experiment are shown above. These products had remarkable storage stability, and were well received during the entire two-year study. Unfortunately, all results are not this good. It can be pointed out, however, that it is possible to produce irradiated meat products which will be stable over long periods of time.

According to our observations, total and intramuscular fat have little influence on stability or preference scores in roast pork.

Table IV  
Mean Preference Scores  
for Roast Pork

Days Storage	Irradiated* 70° F. Storage	Frozen Non- Irrad.Control
0	5.5	5.9
30	5.8	5.9
90	5.7	6.2
150	5.7	5.7
210	5.0	5.9

\*4.5 Mrad

Table IV shows that irradiated pork has fair stability and was only slightly less acceptable than the non-irradiated control up to 150 days' storage; at 210 days, however, there was a decided change in rating.

The principal ingredients in many well received dishes were radiation sterilized chicken, pork, ham, and beef, that had been in storage for periods up to three years, further attesting to the stability of irradiated products.

This briefly summarizes the status of the technology program. It seems clear that by the use of proper processing conditions, acceptable items, which will have good storage stability, can be prepared. Proper processing will involve the selection of the raw material, the method of preparation and enzyme inactivation, the use of selected additives, favorable packaging environments, and in some instances, proper post-irradiation preparation procedures. When adequate information is available concerning all these factors, we will be better able to control adverse radiation induced changes, and can then produce highly acceptable items.

## MICROBIOLOGY OF RADIATION PRESERVATION FOOD PROJECTS

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### Introduction

The most important objective in the radiation preservation of foods is to establish scientific criteria for freeing various foods from inherent microbiological and toxicity problems. Fortunately, vegetative forms of microorganisms, with few exceptions such as Micrococcus radiodurans, need to absorb relatively low doses of ionizing energy to be totally inactivated. Unfortunately, however, ionizing energies have not shown comparable remarkable lethal effectiveness against sporulating bacteria such as Clostridium botulinum.

The process of radiation preservation has been considered in terms of two dose levels; namely, a "sterilizing" (high dose) level (doses in the range of 2 to 4.5 megarad) and a "pasteurizing" (low dose) level (under 1.0 megarad).

The purpose of this presentation is to evaluate the advances which have been made in rendering foods free and safe from spoilage and pathogenic microorganisms and their toxicogenic substances by means of "sterilizing" doses of ionizing energies. The direction of the microbiological research and development program of the Food Radiation Preservation Project has been toward (1) dependence on experience and observations of killing effects of ionizing energies and (2) solution of the riddle of the nature of radiation-resistance in spores of Cl. botulinum.

\*presented paper

Facts in Brief on the Extreme Resistance and Longevity of Bacterial Spores:

The successful application of ionizing radiations to the preservation of foods requires a fuller appreciation and understanding of the fundamental nature of the bacterial spore. This means that there is great need to approach the question of basic information through channels of metabolic activity, the process of metamorphosis of a vegetative cell into a spore and metamorphosis or outgrowth of a vegetative cell from a spore. These and other approaches not yet defined are needed in order to bear down on the virtual indestructibility of the spore in the natural state.

Bacterial spores are remarkable for their extreme resistance and longevity. They can withstand most unfavorable environments of high temperatures, toxic chemical substances, enzyme actions, ionizing energy, high pressures, desiccation, freezing, etc. The spores of some flat-sour microorganisms will withstand steam under pressure at 115°C. for more than three hours. Spores of greatest density are regarded most resistant to heat. Bacterial spores have been isolated from hermetically sealed canned meats after 115 years. Anthrax spores dried on silk threads in Koch's laboratory were found viable after 60 years. How long spores may remain alive in the natural state is not known. The view has been entertained that there is little or no biochemical activity in a spore, since physiological functions depend upon a critical state of hydration. Current concept is that bacterial spores are characterized by a low rate of endogenous respiration. The spore is said to contain a full complement of the enzymes present in the vegetative cell. The spore coat is regarded as being mainly polysaccharide in composition, and may be destroyed by

hydrolysis with hydrochloric acid.

The resistance of the bacterial spore to adverse conditions is said to be due to (1) the impermeability of the spore coat, (2) the peculiar chemical state of the cytoplasm of the spore, (3) the high bound water and lipid content of the spore as compared to vegetative cells, (4) the relatively low salt content of the spore, and (5) spore resistance may be tied up with a higher resistance of spore enzymes due to a postulated enzyme-protein union. Powell of England assumed the resting spore to be a highly condensed waterproof structure. Powell's discovery of dipicolinic acid in resting bacterial spores, later confirmed by Perry and Foster, provided a unique advance in furthering exploration of the character of this remarkably resistant biological form. The remarkable stability of the resting spore indicated an intimate relationship of the constitution of the spore coat and an obscure bonding with a unique chemical substance, calcium dipicolinate. Thus, there has been identified a well-respected biological marker bearing on the problem of bacterial sporogenesis and resistance.

In the numerous investigations of the thermostability of spores, which have followed Powell's discovery, the role of dipicolinic acid has always been implied, creating two opposing schools of thought. One indicates a direct relationship to thermostability and general indestructibility of spores. Another school considers the presence of DPA to be incidental. Proof of any relationship of DPA to thermostability was recently strongly indicated. Church and Halvorson, for example, noted that the viability of aerobic spores decreases as the level of DPA decreases below 1.2 percent. Above this amount there was no correlation. On the

other hand Byrne. Burton and Koch found no relationship of the total amount of DPA to heat stability of anaerobic spores. Workers of West Virginia University report loss of viability and release of DPA are nearly parallel during a three hour interval of heat shock of spores of Cl. botulinum. This suggests that botulinus spores become heat sensitive after releasing a critical percentage of DPA. Recently Walker, Matches and Ayers showed that greater kill was obtained with spores of four different bacillus species which released DPA most readily. Thus, it was shown that the rate of release of DPA during heating appears to be dependent upon or directly relates to the killing effect upon bacterial spores.

The resistance character of the bacterial spore is not settled. Open debate continues. Vinter of Czechoslovakia views thermostability of the complex structure of the spores as a function of a concentration of certain biochemical substances in the spore coat such as calcium and cysteine. He indicates that quantitative differences may be found in the protein structure in the spore coat and vegetative cells. Apparently cysteine and cystine with SS-bridges play an important role in the structure of certain highly inert proteins, such as keratins.

Vinter indicates that the stabilization of keratin-like protein structure by SS-bridges may contribute to the unique character and resistance of the dormant or resting spore. Thus, Vinter is searching for some thermostabilizing biochemical substance in the protein structure of the spore, possibly involving a bonding or other relationship between calcium and cystine-containing protein. On the other hand, Dr. W. G. Murrell of Australia indicates that the bacterial spore coat is freely permeable;

the structure resembling a sponge. However, the core or center of the spore may be impermeable to water. He noted that tritium, for example, readily exchanges in resting as well as germinating spores. Calculations indicate that spores may take up 40% moisture of their own volume when placed in dextran solution. Dr. Murrell, at a recent lecture, indicated he did not believe that heat markedly affected the permeability of the spore coat, and that generally permeability of the spore coat may not be as important in the indestructible character of the spores as is often assumed.

However, advances have been made in our knowledge of the biochemical properties of the bacterial spore. The basic mechanism of the unique thermostability and resistance to ionizing energy appear to be similar. The biological role of the resting spore form is still essentially unresolved. It appears that more basic information and experience are prerequisites, if the problem of the unique resistance of spores to adverse conditions such as the application of ionizing energy to food preservation is to be happily resolved.

In summary, recent important discoveries related to the spore problem indicate that we are slowly rising to a better understanding of its resistance mechanism. However, there still exist wide gaps in our total knowledge of the physical and chemical basis of this resistance.

Progress of Microbiological Studies in Establishing a Scientific Basis for Preservation of Foods by Ionizing Energy:

Since 1954, a number of areas of study were established through the Quartermaster Corps Radiation Contract Program. Research was actively pursued in laboratories of the government, academic centers and the

canning industry. Reliable information has been sought on the problem of the resistance of food spoilage organisms, particularly bacterial spores, to gamma rays. Data have accumulated on physical and chemical factors affecting radio-resistance of spores. It is significant that spores of non-pathogenic and pathogenic species of microorganisms are found to vary in resistance to ionizing energies. We have been particularly concerned with the spore-producing organism, namely, Cl. botulinum, because it forms a highly lethal toxin in foods. The several types, as well as strains within types of Cl. botulinum, vary relatively widely in radio-resistance. It is particularly important, therefore, to know scientifically the lowest dose for killing the spores of the most radiation-resistant strains of Cl. botulinum. Present studies include a search for methods to make the relatively indestructible spores of Cl. botulinum sensitive to killing by ionizing energy.

Physiological studies were needed to shed light on the influence of ionizing radiation on the normal physiological functions of spores of Cl. botulinum. Knowledge so acquired, it was hoped, would lead more directly toward understanding of the precise physiological state of the spores of Cl. botulinum and any eventful biochemical changes during gamma radiation which might be unique to this pathogenic sporulating microbe. From a purely practical viewpoint, we are interested in the general principles involved in the inactivation of spores of Cl. botulinum and the factors governing recovery of radiation injured spores in various growth media. Work in this direction commenced in December 1959 under a project entitled "Studies on the Physiology and

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Metabolism of Spores of Clostridium botulinum" (West Virginia Univ.). In the beginning, the study dealt primarily with methodology. Large numbers of viable spores of Cl. botulinum 62A were produced in a state of synchronous growth. Physiological studies are meaningful when the biological materials are of approximately the same age. A method was developed to harvest crops of "clean" spores, that is, spores free as nearly as possible from adhering contaminating materials and other debris from the vegetative cells and the medium in which they were produced. Success in producing large crops of spores rapidly was achieved by maintaining the vegetative cells of a strain of Cl. botulinum 62A in the interval of its shortest division time, or logarithmic growth phase. The organisms at this state of growth were large in size, grew in chains, and stained uniformly and intensely. Large inocula of synchronously produced vegetative cells were planted in the liter volumes of a simplified trypticase sporulation medium. Sporulation was achieved in more than 90 percent of the cells on overnight incubation. Thus, an important step has been accomplished to provide working materials for application to the technical studies of spore physiology. Funds for this contract have been extended. The study is continuing.

Investigators have indicated that the proposal for a "sterilizing" radiation dose in foods should be equivalent in safety to thermally processed foods. Extensive knowledge has been sought of the resistance of spores of most of 200 known strains of Cl. botulinum Types A and B. Anellis of the QMF&CI has employed for study a model experimental system, in a phosphate buffer solution. The purpose was to 1) obtain baseline

radioresistance data, 2) identify the most resistant spore-forming strains, and 3) determine the relative resistance between the spores. The results indicate that spores of Type A clostridia are probably not any more resistant to ionizing energies of gamma rays than those of Type B strains under the conditions of these experiments. Furthermore, experiments indicate that there is no more than a three-fold difference in resistance of the tested strains of Cl. botulinum of Types A and B. The data indicate that the spores free from surrounding nutrient environments are by themselves relatively sensitive to gamma rays, very few numbers of strains surviving irradiation of 1.4 megarad for a spore load of 10,000 per tube. This information suggests that the radiation dose level requirement for complete killing of spores is not necessarily remarkable and that other factors in the food environment play important roles in enhancing their resistance.

Investigations were made with the view of possibly forecasting effects for practical lowering of the ionizing energy sterilization dose requirement for Cl. botulinum. Under contract with Oregon State College, an investigation has been carried out on the effects of conventional type food additives in lean ground round. The objective was to search for a complementary action of food additives and ionizing energy for enhancing the radiation sensitivity of the spores of Cl. botulinum. The additives explored were salt (NaCl), and spices such as garlic, mustard, cloves, nutmeg, cinnamon and pepper. Experimental tests with the various additives indicate that 3% salt was effective in enhancing killing Cl. botulinum Types A and B at 2 and 3 megarad ionizing energy. Spices appeared to be ineffective, with the exception of mustard (allyl-

isothiocyanate). Mustard, under the conditions of the experiments, promoted the inactivation of C1. botulinum strains A5 and B155 at radiation doses of 1.5, 2 and 3 megarad. These studies are continuing.

A number of interesting findings have been produced through the work of the University of Michigan group. Studies were focused on the combined effects of heat and ionizing energy in food sterilization and on the determination of a radiation sterilization dose. These results are noted. Spores of C1. botulinum show a slight rise in resistance to gamma ray energy at  $-70^{\circ}\text{C}$ . as compared to 0 to  $20^{\circ}\text{C}$ ., at which temperatures the spores were most sensitive. The spores show highest resistance at temperatures of  $40^{\circ}\text{C}$ . to  $80^{\circ}\text{C}$ . or just before thermal inactivation becomes consequential.

C1. botulinum spores inoculated into cooked and raw ground beef and packed in cans in conventional manner have been totally inactivated by use of large doses of irradiation from cobalt 60 gamma energy. Achievement of total sterilization, however, unwittingly disclosed a residual toxigenicity of the food. This toxigenicity may be due either to the spores themselves or to the essentially unchanged enzymatic activity of the radiation inactivated spores. The results of several experiments indicate that 3.8 megarad of ionizing energy were required to totally inactivate  $5 \times 10^6$  spores of C1. botulinum 213B per gram of cooked beef. A dose of 3.72 megarad totally destroyed  $1.7 \times 10^6$  spores of strain 213B per gram of raw meat. The meats from experimental packs inoculated with spores of Type B botulinus culture were subjected to animal tests and found free from botulinum toxin.

Experimental killing conditions were set forth for spores of C1.

botulinum Type A. In cooked beef a load of  $5.2 \times 10^6$  spores of Cl. botulinum 62A was totally inactivated by 3.85 megarad. Raw ground beef, loaded with  $2.67 \times 10^6$  spores per gram, was sterilized at the 3.6 megarad dose. It is significant that all cans of meat experimentally inoculated with more than  $2.67 \times 10^6$  spores per gram tissue proved sterile on tests, but CONTAINED TYPE A TOXIN. The authors indicated that this number of heat-shocked spores by themselves contained sufficient toxin to kill mice. They indicated also that more toxin was present in the experimental cans than could be accounted for by the unirradiated spores alone. The problem of residual toxicity is a laboratory artifact, caused by the use of excessive numbers of spores containing some heat-resistant botulism toxin within themselves. However, the experiments indicate that even in the presence of an overwhelming number of spores of toxigenic clostridia, a dose range slightly less than 4 megarads can eliminate all spores of a relatively indestructible Cl. botulinum species.

A five year "study of the destruction of food poisoning organisms in canned foods subjected to ionizing radiations" has been carried out under a research contract with the National Cannery Association. The applied study was a conjoint effort of several highly competent investigators of three research laboratories associated with the canning industry. A primary objective was the establishment of a minimum sterilizing dose of irradiation in order to provide public health safety. The experimental aspects of the work concerned five foods; namely, corn, peas, pork, beef and chicken soup. Each product was packed in replicates of one-hundred, in 202x202 cans. The foods were "spiked" with a composite

spore suspension of five strains each of Cl. botulinum Types A and B, to a level of 1,000,000 viable spores per container. Ionizing energy was applied in graded doses from none to as high as 4.19 megarad. Irradiation was carried out under conditions of holding the inoculated foods at room temperatures and in the frozen state. Two endpoints of the experimental tests were evaluated; namely, commercial sterility and absolute sterility. Commercial sterility, for purposes of the experiment, was defined on the basis of product spoilage. Absolute sterility was defined as an endpoint of total killing of the most resistant organisms and freedom from spoilage. The results as summarized by these investigators are reproduced in Table 1. It may be observed that gamma ray dose levels showing partial spoilage varied with the foods, but a dose level of 3.72 megarad gamma rays resulted in absolute sterility of all five foods. The experiment showed further that although no toxin was found in the food, a "non-swollen" or non-spoiled can may yield viable spores of Cl. botulinum which on subculture could be induced to grow and form toxin. Thus, based on this information, the conclusion was reached by these workers that despite the fact that viable toxigenic spores remained in irradiated foods at these dose levels, no visible spoilage was observed.

Theoretical considerations showed that at a dose level of nine times the ionizing radiation level capable of effecting a 90% killing, complete sterility would be very likely. The canning industry from long experience with minimum thermal processing techniques for safeguarding health has been guided by the assumption that the minimal processing dose should destroy entirely  $10^{12}$  spores of Cl. botulinum. Assuming killing

mechanisms of thermal and ionizing energy achieve the same end result, it appeared that actual radiation tests with  $10^{12}$  spores of Cl. botulinum would be required to validate this view, although on the basis of the data presented a lesser dose could possibly achieve the requirement of absolute sterility.

Table 1

Commercial Sterility Endpoints in Five Irradiated Foods  
Incubated for One Year at 85° F.

<u>Experimental Food</u>	<u>Radiation Dose (in Mrad)</u>	
	<u>Spoilage</u>	<u>No Spoilage</u>
<u>Room Temperature Condition</u>		
Whole kernel corn in brine	2.88	3.26
Ground, parboiled beef	2.60	2.79
Cream of chicken soup (condensed)	2.60	2.79
<u>Freezing Temperature Condition</u>		
Peas in brine	2.42	2.79
Ground, parboiled pork	3.44	3.72
Cream of chicken soup (condensed)	3.35	3.52

Future Plans

The major problem of establishing a scientific basis for freeing foods from most radiation-resistant toxigenic organisms has been a prime objective of the studies under contract with the Quartermaster Research and Engineering Command. The facts uncovered as result of this research show the spores of Cl. botulinum types to be relatively sensitive

to radiation of about 2 megarad. However, various foods appear to contain substances which either interfere with the killing action of radiation energy or directly impart some protection to spores, raising the radiation-dose requirement for killing in foods approximately two-fold. The support of basic and applied research by the Quartermaster Research and Engineering Command has led to valuable knowledge of radiation-resistance and basic physiology of spores of Cl. botulinum. These studies have contributed much to the knowledge of the character of spores. The direct approach of simultaneous basic and applied research to the sensitive safety question of health, through Cl. botulinum, shows promise of leading us to a better understanding of the spores and to a solution of the dosage requirement for sterilizing by ionizing energy.

There is a need for continued detailed comparative studies of the killing of large numbers of toxicogenic spores in a variety of foods. There is a need to learn more of the biochemical changes in spores of Cl. botulinum during processes of germination or outgrowth and during sporulation. More specific knowledge of the biochemical changes in spores of Cl. botulinum during gamma radiation is needed for clues to the radiation-resistance of the spores. More knowledge is needed of the naturally-occurring numbers of spores in various foods, and particularly, the proportion of spores of Cl. botulinum to spores of non-toxin producing but spoilage inducing spore-forming bacteria. This should aid us in arriving at a more realistic solution of ionizing radiation dose requirements for sterilization of foods.

### Summary

A major task, from the microbiology standpoint, which is still blocking the goal of absolute sterilization by ionizing energies, is the resolution of the character of relative indestructibility of spores of toxigenic Cl. botulinum in foods. Opinion is not crystallized on what the order of the ionizing energy dose should be to achieve total freedom from pathogenic organisms. Data deriving from laboratory tests with overwhelming numbers of spores of Cl. botulinum have not fully satisfied the actual requirement for absolute sterility of foods; however, best indications point to a dosage which may be between 3 to 4 megarad.

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NUCLEAR EFFECTS

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Induced Radioactivity

All food, regardless of where grown or processed, contains a measurable amount of natural radioactivity (Table I). The biological variation of foods is so great that experimentally obtained values of natural radioactive content for supposedly identical food samples vary greatly.

Table I  
Sources of Radioactivities in Food

dpm/lb	curies/gram	Natural	Man-Made	
			Fallout	Sterilization
10 <sup>3</sup>	10 <sup>-6</sup>	K <sup>40</sup> , C <sup>14</sup>		
10 <sup>2</sup>	10 <sup>-7</sup>		Sr <sup>89</sup>	Na <sup>22</sup> (E), P <sup>33</sup> (E), P <sup>32</sup> (E), Rb <sup>84</sup> (E)
10	10 <sup>-8</sup>	H <sup>3</sup>	Sr <sup>90</sup>	Zn <sup>65</sup> (E), I <sup>126</sup> (E), Fe <sup>55</sup> (E)
1	10 <sup>-9</sup>	Ra <sup>226</sup>		P <sup>32</sup> (FE)
10 <sup>-1</sup>	10 <sup>-10</sup>			
10 <sup>-2</sup>	10 <sup>-11</sup>			
10 <sup>-3</sup>	10 <sup>-12</sup>			
10 <sup>-4</sup>	10 <sup>-13</sup>			
10 <sup>-5</sup>	10 <sup>-14</sup>			
10 <sup>-6</sup>	10 <sup>-15</sup>			
10 <sup>-7</sup>	10 <sup>-16</sup>			
10 <sup>-8</sup>	10 <sup>-17</sup>			Sn <sup>119m</sup> (Co <sup>60</sup> )
10 <sup>-9</sup>	10 <sup>-18</sup>			

\*presented paper

After five years or more of research on the problem of food activation, it is clear that activity can be induced in food by radiation processing, the amount being dependent primarily on the energy of the incident radiation used in the processing, and secondarily on the dose which is given the food.

Both experimental and theoretical approaches have been taken in order to clarify the induced activity problem.

The ability to experimentally detect and measure the activity in foods can be affected by:

a. Use of special techniques such as enrichment, concentration (by ashing, etc.) delivering "super" - doses.

b. The selection of the energy to maximize or minimize activation, since activation probability increases with the energy of the radiation used.

c. Use of ultra-sensitive detection equipment and the analysis of extremely large sample batches.

d. The presence in natural foods of those isotopes with high probability of activation.

It has been possible to develop several reliable methods of predicting the amount of activity which might be induced in food by radiation processing. All such methods require detailed knowledge of the source energy, dosimetry, and particularly the isotopic composition of the food samples.

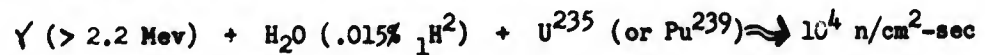
Until recently, this last information was not known to the required degree. During the past year, efforts have been directed toward determining the isotopic content of 4 main meat items to a sensitivity of

1 ppm. This work, in which neutron activation analysis techniques are employed, has resulted in determination of isotopic concentrations for 28 elements.

Whether calculated from theory or actually measured, the process-induced activity in foods is only a small fraction of the naturally present activity level.

Results can best be summarized by radiation source classification.

All experimental work has been completed on measurement of the neutron flux and the activation of food processed in Spent Fuel Rod Gamma Facilities. The experimental data are very plentiful and quite conclusive. All spent reactor rods contain some Lanthanum 140 isotope as well as some unused Uranium 235 or Plutonium 239. Most SFR gamma facilities utilize these rods in a water shield or pool. This water, like all other, is an ideal medium for moderating neutrons and, furthermore contains .015% "Heavy" hydrogen. Thus, in a source utilizing SFR in a water shield we find the following:



Lanthanum 140 is radioactive, and emits gamma rays of 2.51 Mev energy with a very short half life. The deuterium in water can react with gamma rays of energy 2.2 Mev or above, emitting a neutron of 150 Kev energy and becoming ordinary hydrogen. The neutron can now cause one of the atoms of  $\text{U}^{235}$  or  $\text{Pu}^{239}$  to fission with the subsequent production of 2-3 fast neutrons per event. With the entire sequence occurring in a large volume of water all fast neutrons from these fissions are moderated to thermal energies and can regenerate the cycle. In time, an equilibrium condition is established, the SFR source pool becomes a

small reactor, well below criticality, and the neutron flux level stabilizes at, say,  $10^4$  n/cm<sup>2</sup> - sec.

Use of old SFRs means a much smaller amount of Lanthanum 140 and therefor fewer than 2.2 Mev gamma rays to initiate the reaction.

If the SFRs are used in an air medium as at DPG, little or no deuterium is available and there is no moderating medium to slow what few neutrons are produced.

All nuclei produced and observed to date are of very short (15 hours or less) half-life.

Radiation from radioisotope sources such as Cesium 137 or Cobalt 60, or from X-rays, can cause excitation of atomic nuclei which subsequently emit secondary radiation. This is the so-called isomer activation phenomena which occurs only in elements where a large nuclear spin change is possible (Table II).

Exhaustive tests indicate (1) that isomer activation increases with increasing radiation energy, (2) the activity is induced in elements not considered normal food components, and that, (3) at no time during the entire study was induced isomeric radioactivity actually found in samples of whole food or food ash, the amounts detected having been found in special non-food materials included in the test to provide data for theoretical treatment.

The area at present receiving the greatest share of the induced activity effort is that relating to electron beam radiation.

Activation with electrons can be brought about by two mechanisms, the  $\bar{e} - n$  and the  $e \rightarrow \gamma - n$  reactions. Of these the first is the less probable by a factor of  $\sim 1000$ , is a short-range effect, and is not

TABLE II  
SOME ISOMERS OF STABLE ISOTOPES

Isomer	$t_{1/2}$	Level (MeV)	In Foods
1. Cd <sup>111m</sup>	49m	0.40	No
2. Pb <sup>204m</sup>	67m	2.2	10 <sup>-6</sup> %
3. Sr <sup>87m</sup>	2.8h	0.39	No
4. In <sup>115m</sup>	4.5h	0.34	No
5. Ba <sup>135m</sup>	29h	0.27	No
6. Sn <sup>117m</sup>	14d	0.32	2x10 <sup>-7</sup> %
7. Te <sup>125m</sup>	58d	0.15	No
8. Te <sup>123m</sup>	104d	0.25	No
9. Sn <sup>119m</sup>	250d	0.089	2x10 <sup>-7</sup> %
10. Cd <sup>113m</sup>	5.1y	0.27	No
11. Nb <sup>93m</sup>	12y	0.030	No

observed beyond a 2 inch depth in a food package even when the primary electron is of 24 Mev energy (Figure 1).

On the other hand, electron beams will generate Bremstrahlung when penetrating a food package. These Bremstrahlung are X-rays, have great penetrating power, and can lead to a variety of  $\gamma$ -n and  $\gamma$ -p reactions at considerable depth in the food sample (Figure 2).

Such activation is strictly an energy threshold phenomena with each element having a defined probability for activation at each energy (Figures 3 and 4). This threshold situation with electrons (or the X-rays they produce) is being thoroughly examined. All theory and experiment to date indicates the threshold for most elements of  $A \leq 60$  is 12 Mev or more (Table III).

SGO and QMC contractors working independently and using extremely sensitive detection devices have thus far established that:

a. Sterilization with electrons of 16 Mev energy and above produces minute but detectable radioactivity in food. Activity is of both long and short half-life type (Figure 5).

b. Sterilization with electrons of 12 Mev energy produces no detectable radioactivity in food.

The threshold region 12 to 16 Mev is now being very actively examined with the intent to positively locate the energy threshold and probability cross-section for all isotopes known or suspected of being present in foods.

I should like to comment on other aspects of our induced activity effort.

The total number of foods, by type, consumed by the average

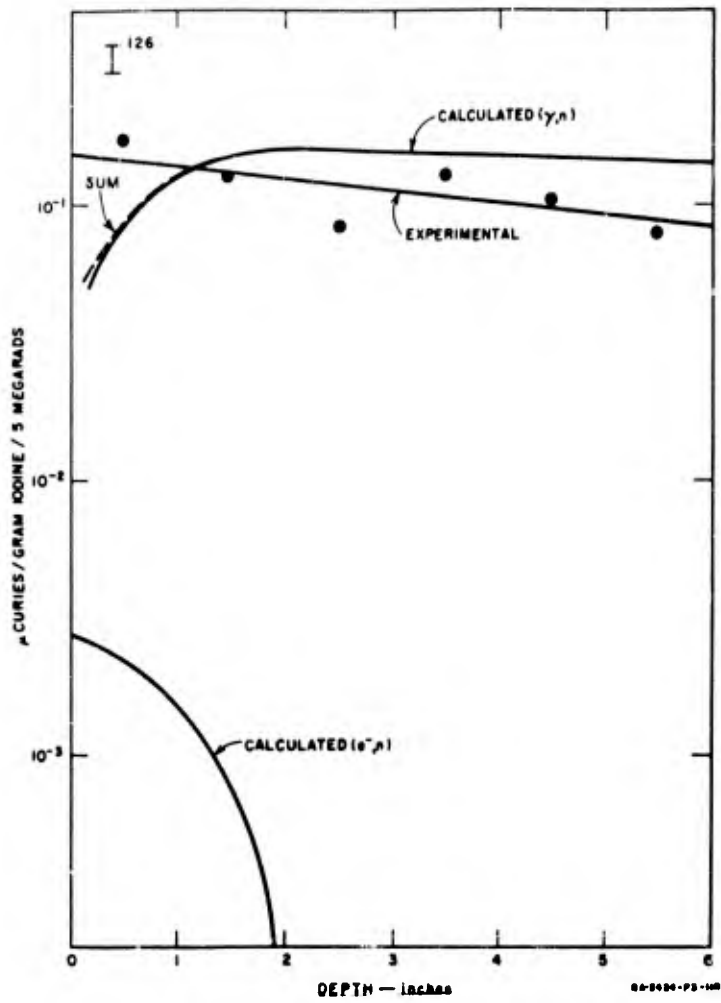
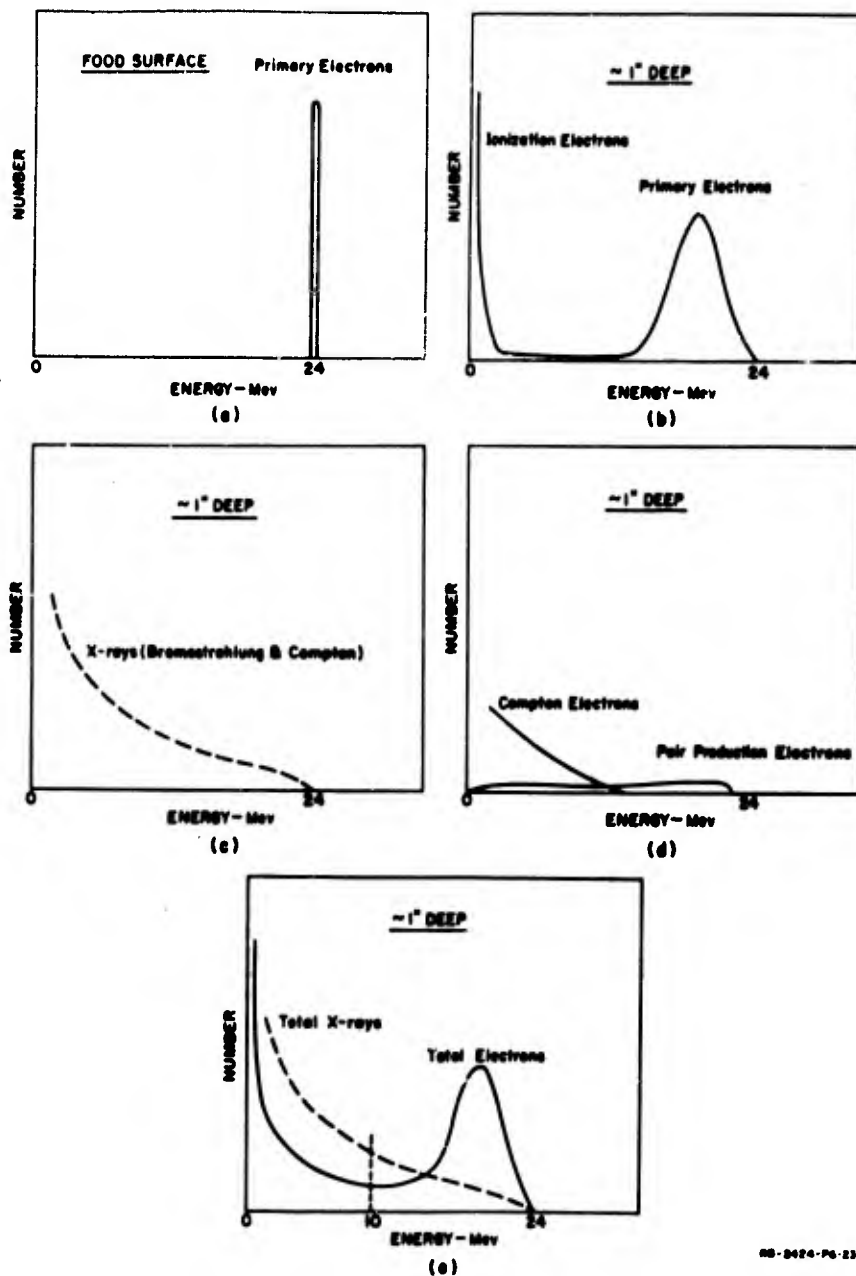


Figure 1.

HAND CALCULATION OF  $I^{126}$  PRODUCTION IN BEEF



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Figure 2.

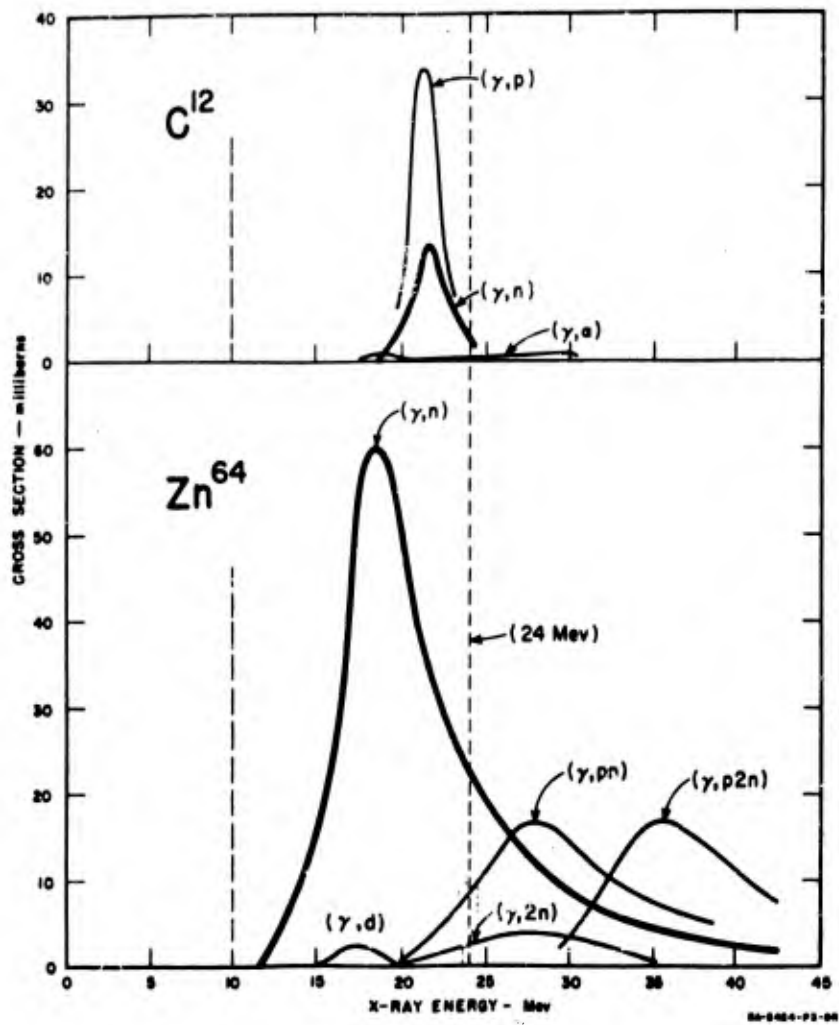
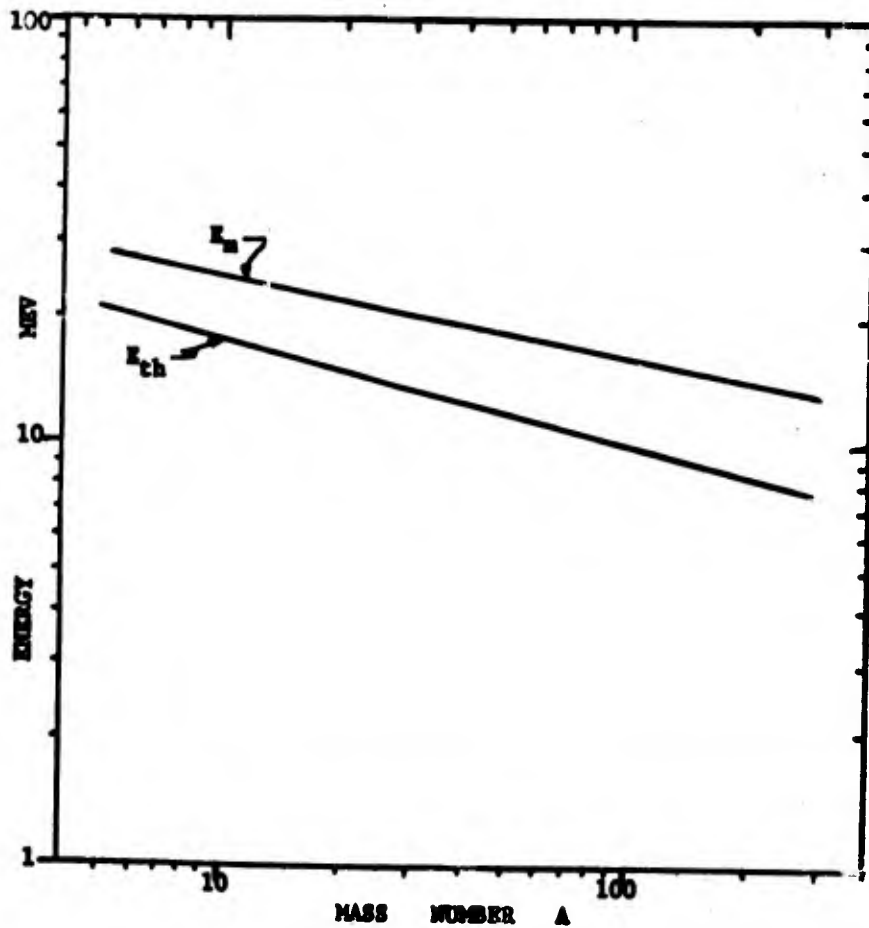


Figure 3.



Plot of  $E_n$  and  $E_{th}$  for the  $(\nu, n)$  reaction as a function of Mass Number. The line shown for  $E_{th}$  can be well represented by  $E_{th} = 32 A^{-0.27}$ .

Figure 4.

TABLE III.

## Threshold Energy Levels for the Production of Induced Radioactivity

Element	Type of Nuclear Change	Threshold Energy (Mev)	Half-Life of Product
Li <sup>7</sup>	( $\gamma$ , p)	9.8	0.85 sec
C <sup>12</sup>	( $\gamma$ , n)	18.7	21 min
N <sup>14</sup>	( $\gamma$ , n)	10.65	10 min
O <sup>16</sup>	( $\gamma$ , n)	16.3	2.1 min
Na <sup>23</sup>	( $\gamma$ , n)	12.1	2.6 y
Mg <sup>25</sup>	( $\gamma$ , n)	11.5	14.8 h
Al <sup>27</sup>	( $\gamma$ , n)	14.0	7 sec
P <sup>31</sup>	( $\gamma$ , n)	12.35	25 min
K <sup>39</sup>	( $\gamma$ , n)	13.2	7.5 sec
Ca <sup>40</sup>	( $\gamma$ , n)	15.9	1 sec
Fe <sup>54</sup>	( $\gamma$ , n)	13.8	8.9 sec
Cu <sup>63</sup>	( $\gamma$ , n)	10.9	10 min
Zn <sup>64</sup>	( $\gamma$ , n)	11.8	38.3 m

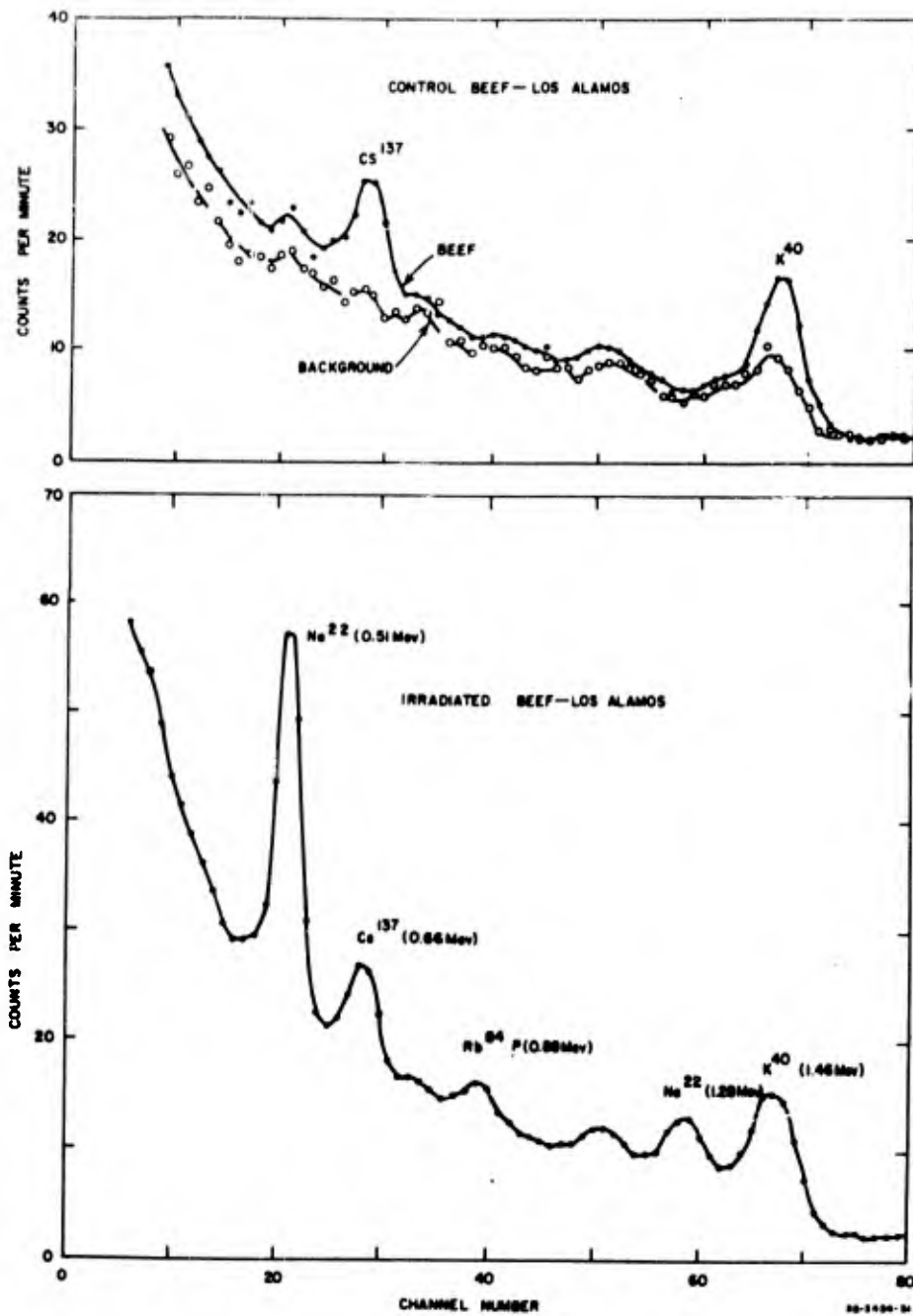


Figure 5.

individual is far too great to permit detailed experimental evaluation of the induced activity in each. Nor is such an experiment justified, since many items of the diet are not considered likely candidates for radiation processing. On the other hand there are obvious advantages to obtaining an experimental value for that maximum amount of activity which would occur in a total irradiated diet. To this end a composite menu, relatively free of biological variation, blended to insure homogeneity, and including normal portions (as consumed) of all line items making up the Annual General Menu, has been developed and a sizable amount of this sample mix prepared for use. This will reduce the requirement for detailed investigation of each separate food item, and provide the activity value associated with a 100% irradiated diet. We have encouraged other investigators working on the induced activity problem to work with some of this mix so that cross-comparison of results and techniques can be accomplished.

The QM work has dealt with measurement of radioactivity in food or food like materials. No effort has been made to detect the amount of activity likely to become part of body tissue through consumption.

#### Dosimetry and Dose Distribution

At the outset of the program of radiation preservation of food in 1954, very little was known of high intensity dose measurement and within the program no one could predict with any degree of certainty what would ultimately prove to be the sterilization, pasteurization, deinfestation, or sprout inhibition dose ranges. Consequently dosimetry and dose distribution efforts, from the beginning were, by design, directed toward

finding measuring devices and techniques capable of being used in any or all of the four dose range applications.

The tasks associated with dosimetry (measurement of radiation dose at a point in space) and dose distribution (dose measurements throughout food sample volumes) proved to be among the most difficult and challenging in the program due to the following complicating factors:

1. Wide range of dose requirements ( $10^4$  to  $10^7$  rad).
2. Wide range of radiation energies used:
  - 0.3 - 3.0 Mev or higher for gamma - X-rays
  - 1.0 - 50.0 Mev for electrons
3. Variable dose rate required
  - to  $10^7$  rad/hr for gamma - X-rays
  - to  $5 \times 10^8$  rad/min for electrons
4. The scattered geographic locations, and considerable variations in source geometry and routine dosimetry techniques preferred by source operators

The prime function of any dosimetry system, whether used to measure a sprout inhibiting, pasteurizing, or sterilizing dose, is to ascertain that an accurate and precise dose is delivered to a food sample. To accomplish this, it was foreseen that four general kinds of dose measuring devices would be required. These functional types are:

1. A "primary standard" dosimeter against which all other dosimeters used in the program can be checked and calibrated.
2. A secondary dosimeter, which serves as the basic standard for a particular source geometry, and is specifically suited for the operating conditions of that source.

3. A simple, inexpensive, easy-to-read "GO-NO-GO" dosimeter which would be affixed to each sample during irradiation, so as to guarantee that the sample receives no less than a critical minimum nor more than a limiting maximum dose.
4. For electron accelerator sources, a beam monitor which would measure various electronic parameters from which accurate calculation of dose delivered could be made.

To develop a dosimetry capability to meet the need of the QMC, efforts were directed along several lines:

1. Aid was solicited and obtained from the NRC and other national committees in the testing and standards fields;
2. In-house, contract, and working agreement research was conducted aggressively;
3. Information and data on dosimeter development was solicited in exchange for QMC assistance and counsel on dosimeter problems;
4. Proven dosimeter systems were purchased as available.

The QMC effort in the dosimeter field resulted in the development, by research and adaptation, of an integrated family of dosimeters. This dosimeter family, or system, is by no means the ultimate; yet it is, we believe, a usable system from which, with diligent effort, an improved system or systems can and will evolve. I should like to describe some aspects of this system to you.

The Fricke dosimeter was selected as the primary standard for the calibration of other dosimeters, since with proper handling it can be accurate to  $\pm 1\%$ . However, this dosimeter is only usable up to the

range of 50,000 rad, so other systems were sought for the Megarad dose region.

The ceric dosimeter is one of two secondary standard dosimeters used by the Quartermaster Corps today. The mechanism of the ceric sulfate dosimeter had been extensively studied by radiation scientists for a number of years, and since it worked well in the Megarad region, its adoption for high dose ranges was very natural. Ceric sulfate is not an easy chemical system with which to work because of its sensitivity to impurities and, therefore, considerable effort was necessary to minimize this sensitivity. During the past year and a half, the use of pharmaceutical techniques for filling, sealing and ultimate analysis of the ceric vials improved stability and increased the accuracy of these dosimeters. Today, with care, we can achieve  $\pm 2\%$  accuracy.

The other secondary standard dosimeter, cobalt blue glass, is usable in the dose region from about 50,000 rad to 1 Mrad. This dosimeter has served particularly well for dose distribution measurements and electron beam dosimetry. However, it has some limitations. It is accurate to about  $\pm 10\%$  and the fact that, unlike the aqueous solutions used in the Ceric or Fricke systems, its atomic composition is quite different from that of food qualifies the use of this glass dosimeter. Also, the melt is not homogeneous and so, to achieve reasonably accurate results, the light absorption of each chip should be read prior to irradiation and not just an average value assumed.

In the past two years a great deal of work has gone into the development of the polymerization type go-no-go dosimeter. This

dosimeter has proved to be accurate in tests studying such factors as storage stability, temperature effects and dose rate response. The newer dosimeters are quite accurate, ( $\pm 5\%$  on an individual basis), and  $\pm 2\%$  if a large enough group is exposed at one time to give a statistical base for calculation.

Another dosimeter which, while not truly fitting into the production dosimeter picture, has been extensively tested and used in special dosimetry applications is blue cellophane. The thinness of this film dosimeter has made it unique for minute dose distribution studies; however, its non-uniformity of composition and its moisture content dependence has considerably limited its use.

A secondary electron beam monitor was completed this winter and is now installed on the HVEC-ARCO linac at Rockford, Illinois. This monitor actually has seen but limited service to date.

A graphite ionization probe, designed and built for the QMC by contract, was calibrated at the Bureau of Standards last year, and has seen service at the Cook Electric and Argonne National Laboratories cobalt-60 sources.

In order that an up-to-date and adequate high level dosimetry system will be available for the initial testing and subsequent operation of the QMRL, we are having our original dosimetry survey brought up-to-date this next year. As a by-product of this survey a dosimetry handbook will be prepared which will describe in detail the use of three or four selected dosimetry systems that will best serve the needs of the QM.

The spatial distribution of absorbed dose, i.e., the distribution

of the energy absorbed from the radiation field per unit mass of food is as equally important to the food processor as an accurate system of dosimetry. Since the uniformity of food treatment is dependent upon the distribution, it is desirable, ideally, that it be completely uniform. Absolute or complete uniformity cannot, however, be attained in practice, for the energy absorption process, on which the radiation treatment depends, causes the intensity of the radiation field to vary throughout the food. The problem becomes then one of developing irradiation and measurement techniques that can produce the most uniform dose distribution in a food package.

Distribution can be measured by inserting small dose sensors at various points in the food samples prior to processing and then withdrawing and analyzing these monitors afterwards. The disadvantages of this procedure are obvious both from an operational and a product quality standpoint.

A more realistic solution is to measure the radiation parameters, such as energy, current and dose rate which can be related to absorbed dose.

The relationship between these parameters and absorbed dose is established by means of dosimetric test packages throughout which are distributed a number of dosimeters. These test packages, or phantoms, are constructed to simulate, as closely as possible, energy absorption characteristics of the various types of production packages. These phantoms are then irradiated using the same parameter values, radioisotope (or beam energy and beam current), dose rate and conveyor speed that will be used during the production process. As additional

assurance of satisfactory operation, test packages are interspersed periodically in the production run.

One problem in using scanned electron beams is that each pulse must be monitored to insure that every part of the food receives the desired dose. If there is any question, the package must be rejected. Since the characteristics of the beam will change somewhat each time it is turned on and off or its electrical parameters are varied, it seems very desirable to have a three-dimensional phantom of electrically sensed transducers that can be slipped in and out of the beam to align it for each run. The outputs of the transducers can be displayed simultaneously on multi-channel oscilloscopes in the control room and the controls of the linac can be varied until a desired and precise pattern is achieved. The phantom can then be moved out of the way by remote control, and food packages run in front of the beam without it ever being turned off. Such a phantom system should insure that much more reliable irradiation is done much faster.

The Quartermaster Corps has established that to achieve the desired biological effect with adequate wholesomeness, the radiation energy received by a sample at any point should be within the limit of 100-115 per cent of the prescribed dose.

To decrease dose variation, techniques such as bidirectional radiation have been used. Further uniformity is achieved by the use of absorbers and scatterers placed on the top, bottom, and sides of the food packages.

Experimental studies of the dose distribution as a function of select process variables such as radiation energy, sample composition, and

container configuration are necessary for all samples.

Special experimental dosimetric effort is also needed and underway, to investigate heterogeneous sample systems and secondary electron doses at container-food boundaries. Such studies now being conducted, when given proper analytical treatment, are vital to the achievement of adequate dose and dose distribution particularly in specialized processing techniques such as large volume sample irradiations.

## PACKAGING FOR IRRADIATED FOODS

George E. Tripp  
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In the beginning of this program we were faced with the problem of finding satisfactory containers for foods sterilized by radiation in the least possible time. As a consequence, the initial work was concentrated around the most advanced type of container, the tin-plate can. As work progressed, increasing emphasis was placed on less advanced type containers, such as plastic laminations. During the course of the investigations various container materials and container types were considered. Many were placed on the shelf for future reference because problems associated with them did not appear amenable to rapid solution. Now that we are reaching our initial goal we will be able to reconsider these items. We expect that with suitable modification many of these can be used, thereby broadening our base of supply. In this regard we would not be surprised to find that some of the items which were placed on the shelf prove superior to those which we initially chose as our "best bet."

### Tin-Plate Cans

The tin-plate can has been with us for a long time and has achieved an enviable record for low cost, rugged, dependable protection. As simple as the tin-plate can may seem, it is in reality a complex package varying in type of steel, tin-coating weight, interior enamel and end sealing compound. Each food has its own requirements; however, we are fortunate that many foods have similar requirements, thereby limiting

many possible combinations.

Packaging of irradiated foods would be simplified if the tin-plate cans in use for their commercial thermal processed counterparts were satisfactory. Therefore, we needed answers to the following questions:

1. Are commercial type steels suitable for irradiated foods?
2. Are commercial tin coating weights satisfactory for irradiated foods?
3. Are commercial interior enamels satisfactory from the standpoint of service shelf life and extractives?
4. Are commercial type end sealing compounds suitable for irradiated foods?
5. Does radiation processing promote tin rot?

Over the years the Can Manufacturers Institute and various can companies have accumulated a considerable body of data relative to tin-plate cans for thermal processed food. Therefore, in finding the answers to questions 1. through 3. above, it seemed desirable to design experiments so that we could use this data. A well designed experiment with a few products might enable us to predict the behavior of untested items with a reasonable degree of confidence. Two such experiments have been conducted<sup>1,2</sup>, encompassing six types of foods, each representative of a food class. Based on these studies it is evident that the base steel and tin-coating weight used for the thermal processed item will be satisfactory for the irradiated counterpart. Interior enamels have been somewhat troublesome; however, three commercial enamels (Table I) give satisfactory performance and provide us with a best and

second best choice for virtually all irradiated items. The polybutadiene formulation is of particular interest since it is an almost universal enamel. By proper choice of base steel, tin-coating weight and the polybutadiene enamel we will be able to use one can in packaging a wide range of products such as would occur in pilot plant type production.

The problem of enamel extractives has received attention<sup>2</sup>. The quantity of extractive material observed during the irradiation process does not differ greatly from that observed during thermal processing (Table II). Further, infrared spectra of the various processed enamels did not demonstrate any significant differences between various treatments. The infrared data must be used with caution since some changes may have been too small to be detected by this means. Additional, less direct, evidence of the non-harmful nature of enamel extractives was made available under the Wholesomeness Program. During the animal feeding studies a wide variety of tin-plate cans, some plain, some enameled, were used for the food, with no evidence that the interior enamels were harmful.

The most important component of tin-plate cans for sterile foods is the small amount of gasketing material, known as compound, applied to the can end. It is this compound which assures hermetic closure of cans. During irradiation, compounds based on unvulcanized butadiene-styrene rubber, butadiene-acrylonitrile rubber or natural rubber are cross-linked and toughened<sup>3</sup>. Compounds based on neoprene or vulcanized rubber are not noticeably changed. Butyl rubber based compounds are softened and therefore are not satisfactory for irradiated food cans.

Our concern over problems such as tin rot may appear as though we

are going to extremes to find trouble. However, when one thinks of the millions of dollars which will be invested in military supplies, caution is essential. Tin exists in three forms, the alpha or gray form, stable below  $18^{\circ}$  C, the beta or ordinary form, stable from  $18^{\circ}$ - $170^{\circ}$  C, and the gamma or brittle form stable over  $161^{\circ}$  C. Pure tin may change from the beta to the alpha form at temperatures below  $18^{\circ}$  C; at lower temperatures the likelihood of conversion increases. Temperature alone is not sufficient to induce the change, other factors such as scratches, stress or unknown conditions must exist before the transition can occur. Once the conversion starts, the entire mass will, by crystal growth, alter to the alpha form. This is quite serious since the ordinary silvery form becomes a fine powdery material, rendering the tin coating ineffective in protecting the base steel. Although the tin used in coating steel is very pure, we have never encountered this condition in the Military Supply System. However, radiation creates dislocations in metals and other crystal lattices. In the case of tin, the dislocations, which persist for long periods, may provide the necessary conditions for tin rot. Current evidence, however, indicates that the beta-alpha transition will not be a problem. Irradiation of high purity tin and storage at low temperatures did not influence the beta-alpha transition. Further, we have irradiated many cans containing frozen food, and stored them at freezer temperatures without encountering any difficulty.

Three unusual properties of irradiated food have been observed. These are: the production of quantities of hydrogen gas; an increased dezincing effect and a decrease in sulfide staining of the tin-plate. The production of hydrogen gas (Table III) is troublesome since it

obscures shelf life test data and also because bulged cans may be interpreted as evidencing spoilage. An equation<sup>2</sup> has been developed relating initial headspace vacuum, headspace, and the type and quantity of food with the residual vacuum after irradiation. By use of this equation we are able to predetermine the headspace and vacuum necessary to insure the desired vacuum after processing. Unfortunately, this requires that cans be underfilled. Some considerations have been given to dry packing foods such as peas, string beans, corn, cherries, etc. and in this manner obtain the necessary headspace as interstices. This practice is not recommended if very uniform dose distribution is desired. Franke<sup>26</sup> has reported on irradiation photographs of nonhomogeneous layers. A layer of peas or a "weighted" textile fabric shows an irregular distribution of spheres, or an approximate cross lattice, respectively, in the irradiation photo. In a few superimposed layers of this type, some overlappings occur whereby the form in the silhouette is lost. With a large number of layers (20 layers of peas, 100 layers of fabric) the form practically returns. The number of threads in the fabric is as easily distinguished in 100 layers as in one layer, with the exception that the picture is slightly diffused. Correspondingly, the irradiation photo of a rubber sponge with approximately 50 hollows in the path of the ray had almost the same appearance as an optical image of its surface. Laue<sup>27</sup> has investigated this on a theoretical basis. His considerations show that the relative intensity differences in a large number of layers are not canceled, their average is even increased. These findings are of importance when irradiating foods. For example, if we irradiate dry packed peas and assume that the average

density will determine the dose distribution we may underdose a portion of the product by as much as 10%. On the other hand if we consider that a solid column of peas will exist and determine the dose on this basis we could overdose a quantity of the product by the same percentage. The best way to avoid this problem is to fill the voids with liquid, i.e. brine water or syrup, having a density similar to that of the product.

Irradiated foods exhibit marked dezincing properties; that is, they tend to remove zinc oxide pigments from interior can enamels. This problem can be overcome by eliminating the pigment during formulation of the enamel. Omission of the zinc oxide is not serious since the sulfur compounds present in irradiated foods do not react with the metal surface of the can as readily as sulfur compounds in thermal processed foods.

#### Aluminum Cans

Aluminum cans are of interest because of their low absorption density. Investigations<sup>4</sup> indicate that there are no insurmountable problems with the aluminum or interior enamels (Table IV).

The production of hydrogen gas and attendant bulging is a serious problem since the physical strength of aluminum cans is such that they cannot withstand as high an internal vacuum or pressure as the tin-plate steel can.

#### Flexible Plastic Containers

Flexible plastic containers, because of their savings in weight and cube, have an important place in the Military Supply System. Further, their low absorption density and ease of manufacture in a wide range of shapes and sizes makes them attractive for use with irradiated foods.

Several problems arose which required resolution before containers of this type could be used for irradiated foods:

1. Does the food have an adverse effect on the package which might impair its serviceability?
2. Does the package have an adverse effect on the food?
3. Will the package provide adequate protection against physical abuse?
4. Will the package provide adequate protection against microorganisms and insects?
5. Will free radicals, formed in the package during irradiation, react at some subsequent time, affecting the resolution of the above questions?

Effect of Foods on Package: Plastic type packages have been in use for foods since the mid 30's. Since 1945 increasing use has been made of packaging of this type. Therefore, at the inception of this program we had considerable experience in packaging frozen and refrigerated as well as non-perishable foods. This experience had given us considerable insight into the food-package relationship. With this background as a starting point we initiated experiments<sup>5,7,9,10,11</sup> with aqueous solutions (stress-cracking agents) representing foods. In general, the effects were similar to those observed with normal refrigerated and non-perishable items. However, in the course of this and other work<sup>6,8</sup> we encountered a large number of package failures, particularly at the sealed areas. This stress-cracking phenomena was studied extensively<sup>5</sup>. Table V demonstrates the effects of various foods in promoting, and the influence of radiation cross-linking in retarding stress-cracking.

Irradiation improves the stress-cracking resistance of polyethylene whereas it has little influence on the resistance of polystyrene. Table VI shows the response of various polyethylenes to stress-cracking and to radiation. Currently, it is possible to select the species of polyethylene having the greatest stress-crack resistance, and with additional improvement occurring during radiation, this problem disappears.

Polyamids, polyesters, polypropylene and high impact polystyrenes are not troublesome in this regard.

Effect of Package on Foods: During studies in this area<sup>6,12,13</sup> changes were observed in food color, appearance and keeping quality. However, these effects were of the magnitude and extent that would have been anticipated on the basis of moisture vapor, transmission, oxygen transmission rates and light transmission. Foods packaged in plastic type packages containing an aluminum foil ply were generally satisfactory.

Workers in other fields have demonstrated that polymers would undergo chain scission during irradiation. Since the low molecular weight fragments produced could impart off odors and flavors, this subject was explored. Various polymers have been evaluated by subjective odor evaluation<sup>14</sup> (Table VII). The odor intensities of irradiated polystyrene and nylon 6 are quite low while the odor intensities of some types of irradiated polyethylene are quite intense. The composition of the gaseous products has been explored<sup>15</sup> and Table VIII summarizes a part of this data. Extractive substances have also been observed<sup>5,16,17</sup> (Table IX).

Recently an additive has been developed for polyethylene, which

markedly reduces the odor and extractive substances<sup>18</sup>. A quantity of polyethylene containing the additive is now being produced. If the additive proves successful in commercial tests we may have this problem well in hand.

Effect of Radiation on Physical Properties: The capacity of a package to withstand rough handling depends upon numerous physical properties such as tensile strength, elongation, strength of seals and tearing resistance. In general, the effect of radiation on the tensile strength, elongation, heat seals, and moisture and gas transmission rates of plastics and plastic laminations is small<sup>19,20,21,22</sup> (Table X).

However, in the case of cellulosic type materials we note a decrease in elongation, tearing resistance and flexure resistance of approximately ten percent<sup>21,22,23</sup> (Table XI). In the latter case we can compensate for this decrease by using heavier weights of papers and cellulosic type packages.

Latent Effects: During the irradiation process free radicals are produced in the plastic package. There is a distinct possibility that during storage free radicals could react having an adverse effect on the package. Investigations in this area are complicated by changes which normally occur during storage. Studies in this area<sup>5,6,7,8,13,19</sup> indicate that changes occurring in storage are those one would normally expect. If latent radiation effects are present, their influence cannot be detected by conventional techniques.

Penetration by Microorganisms: When contemplating flexible type containers for sterile foods we must also consider the possibility of microorganisms penetrating the package wall and seals. During

manufacture of flexible materials faults and imperfections are encountered. The fault count is related to the thickness of the material (or plies) and the number of plies. To a lesser degree the manufacturing technique will also influence fault count. Some additional damage will occur when the material is fabricated into a package, and the filling and sealing operations will also contribute to imperfections. Further, we must consider the possibility of damage during shipping and storage of supplies. It should be noted that this application requires the utmost in performance, since one hole, permitting entry by one microorganism, can compromise the package's integrity.

Studies<sup>24</sup> have demonstrated that very thin films (less than 0.001 inch) have sufficient imperfections to preclude their use. Films 0.001 to 0.003 inch thick are proof against microorganisms, however, simple creasing will damage them to the extent that they are not satisfactory. Films over 0.003 inch appear satisfactory.

Radiation does not affect the penetration of microorganisms and heat seals which are carefully made appear to be impervious to them.

The resistance of flexible containers to rough handling is under study<sup>17</sup>. Table XII shows how various types of damage affect representative flexible containers. In these tests, failure was determined by penetration of dye solutions. Recently, we have found that this technique is not reliable. With very small holes, surface tension is capable of preventing dye penetration; further, laminates containing a thick ply (such as 0.003 inch thick vinyl laminated to 0.00035 aluminum foil laminated to 0.0005 polyester) exhibit an apparent self-healing property. The self-healing is not genuine however, since small

pressure differences can reopen the hole and permit penetration.

Insect Resistance of Packages: In addition to rough handling hazards, packages are faced with attack by insects. At present, without some form of chemical treatment, no flexible container is completely impervious to all insects. Further, there is no way of knowing whether insects will attack a given package; insects have been known to attack packages even though the contents were useless to them. Investigations<sup>25</sup> have demonstrated that irradiation doses in excess of one megarad will destroy the repellency of pyrethrin-piperonyl butoxide treatments. As a consequence, this treatment would have to be applied after irradiation or the packages packed into an insect resistant treated case liner after irradiation.

#### Summary

Tin-Plate Cans: The commercial type steels and tin coating weights used for thermal processed foods are satisfactory for the irradiated counterpart. Commercial end sealing compounds, except those formulated with butyl rubber, are satisfactory. Three commercial interior enamels give satisfactory performance for all classes of irradiated foods and provide us with a "best" and suitable alternate choice. These are formulations based on polybutadiene or epoxy-phenolic or oleoresinous type resins. The polybutadiene is of interest since it comes closest to being a universal enamel and permits us to use a universal can for limited production where a wide range of items are being processed.

In respect to enamel extractives, the amounts observed during the irradiation process do not exceed that observed during thermal processing.

Aluminum Cans: Work with aluminum cans is progressing. To date we have not encountered any serious problems.

Flexible Plastic Containers: Extensive investigations with various polymers demonstrated that polyethylene had most of the characteristics desired as the inner ply of laminated plastic packages. The chief obstacle to the use of polyethylene was the release of gaseous and extractive substances during irradiation. An additive has been developed which reduces the odor and extractive formation. Currently, the additive is being evaluated on a commercial production basis. If results with the additive continue to be successful, a package consisting of polyethylene-polyester-aluminum foil-tissue will be satisfactory for irradiated foods.

Remaining Problems:

1. Clearance of interior can enamels by the SGO and Food and Drug Administration.
2. Clearance of polyethylene with additives by the SGO and Food and Drug Administration.
3. The current flexible container is over-engineered. The reason for this is the need for considerable caution in introducing packages of this type for sterile food items. We need information in respect to rough handling characteristics of packages of this type so that we can reduce the material requirements and the cost.
4. We need to re-examine the materials which were temporarily placed on the shelf to determine if we can develop an improved package or broaden our base of package supply.

Table I  
 Can Enamels Found Satisfactory for Irradiated Foods  
 After 12 Mo. Storage at 98° F.

Product	Enzyme Activity	Irradiated Product		Thermal Processed Product	
		Best	Alternate	Best	Alternate
Cherries	Inactive	Polybutadiene	Oleo-resinous	Polybutadiene	Oleo-resinous*
	Inactive	Polybutadiene	Epoxy-Phenolic	Polybutadiene	Oleo-resinous
Beef	Inactive	Polybutadiene	Epoxy-Phenolic	Polybutadiene	Epoxy-Phenolic*
	Active	Polybutadiene	Epoxy-Phenolic*	---	---
Codfish***	Inactive	Epoxy-Phenolic	Heat Reactive Phenolic	Epoxy-Phenolic*	Heat Reactive Phenolic**
	Active	Epoxy-Phenolic	Heat Reactive Phenolic	---	---
Pork	Inactive	Polybutadiene	Epoxy-Phenolic	Polybutadiene	Epoxy-Phenolic
	Active	Polybutadiene	Epoxy-Phenolic	---	---

\*Borderline performance  
 \*\*Sulfide discoloration on product  
 \*\*\*Polybutadiene not tested with this product

Table II  
Weight Changes in Enamels

Enamel	Extraction Medium	Sample Number	Weight Change (mg)		Calculated PPM Extracted Enamel Per #2 Can	
			Irradiated	Thermal	Irradiated	Thermal
Oleoresinous	Water	1	-3.4	-16.2	1.9	9.0
		2	-3.0	-15.6	1.7	8.7
	Citrate-Sucrose	1	-29.5*	-21.5*	15.4*	11.9*
		2	-25.9*	-14.8*	14.4*	8.2*
Polybutadiene	Water	1	+15.8	-6.8	Gain	3.8
		2	+17.2	-7.2	Gain	4.0
	Lard-Salt	1	+9.8	-0.7	Gain	0.4
		2	+9.6	+0.4	Gain	Gain
	Citrate-Sucrose	1	-15.4*	-8.5*	8.5*	4.7*
		2	-16.3*	-14.0*	5.0*	7.8*
Epoxy-Phenolic	Water	1	+5.0	+5.2	Gain	Gain
		2	+1.8	-1.4	Gain	0.8
	Lard-Salt	1	+0.9	+0.7	Gain	Gain
		2	+1.8	+1.3	Gain	Gain

\*Corrected for detriming. These values would show weight gains, not losses.

Table III  
Headspace Gas

Product	Irradiated cc/gm	Thermal Processed cc/gm	Increase on Irradiation cc/gm
Beef <sup>1</sup>	0.012	0.005	0.01
Green Beans <sup>2</sup>	0.038	0.020	0.017
Codfish <sup>1</sup>	0.038	0.010	0.028
Pork <sup>1</sup>	0.138	0.053	0.085
Cherries <sup>1</sup>	0.128	0.031	0.097
Chili Con Carne <sup>1</sup>	0.142	0.021	0.121
Sucrose Solution <sup>3</sup>	0.165	0.013	0.152

1 - dose 6 Mrad  
2 - dose 3.25 Mrad  
3 - dose 2.8 Mrad

Table IV

Summary of Aluminum Can Storage Data  
6 Mo. Storage at 100° F.

Product	<u>Irradiated</u>		<u>Thermal Process</u>	
	<u>Enameled Interior</u>	<u>Plain Interior</u>	<u>Enameled Interior</u>	<u>Plain Interior</u>
Whole Kernel Corn	S	S	S	S
Green Beans	S	S	S	S
Ham (Phosphate)	S	S	S	S
Tuna	S	U	S	U
Peaches	S	U	S	U

S - Satisfactory  
U - Unsatisfactory

Table V  
Foods as Stress-Cracking Agents  
(Hours to Failure)

	Dose Megared	0.5% Igepal CO-650	0.5% Lactic Acid	Ground Beef Slurry	Ground Pork Slurry	Fish Slurry	Green Bean Slurry	Apple Slurry	Homo- genized Milk
Polyethylene d.-0.945-0.96 M.I.-0.5 Mn.-30,000	0	24-72	288-308	—	—	—	—	—	—
	1.0	72-90	288-308	336-504	336-504	336-504	0-168	0-168	168-336
	3.0	284-304	N.F.	1512-1680	840-1008	1008-1176	672-840	1344-1512	1176-1344
	6.0	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.	N.F.
Polystyrene Medium shock resistant	0	N.F.	N.F.	—	—	—	—	—	—
	1.0	N.F.	N.F.	840-1008	0-168	168-326	N.F.	1176-1344	0-168
	3.0	N.F.	N.F.	840-1008	0-168	N.F.	N.F.	840-1008	0-168
	6.0	N.F.	N.F.	326-194	0-168	168-326	N.F.	840-1008	840-1008

N.F. Means no failures after 2,000 hours at 122° F



Table VII

Effect of Irradiation on Odor Intensity\* of Packages

Package	0 megarad	1 megarad	3 megarad	6 megarad
Nylon 6	1.9	1.4	1.7	1.4
Polystyrene	3.0	2.4	2.6	1.6
Polyester (type G)	1.6	2.5	2.4	2.1
Polyester (type D)	2.2	2.0	2.6	2.5
Polyethylene (high density Ziegler process)	1.7	3.8	3.6	3.9
Polyethylene (no additives)	1.6	3.2	3.6	4.7
Wax (Fischer Tropsch)	2.0	3.7	4.6	5.3
Polyethylene (6.0% Carbon black plus antioxidant)	2.6	4.4	5.2	5.4
Polyethylene (commercial film)	1.4	4.0	5.2	5.6
Polyethylene (2.5% Carbon black plus antioxidant)	1.6	4.2	5.5	6.0

\*Odor intensity rated on a scale of 0 to 9

Table VIII  
 Gaseous Products at Six Megarad  
 (Micromoles per Gram)

	Polyethylene (Low Density)		Polyethylene (High Density)		Fluoroethane		Polyester*	
	Vacuum	Air	Vacuum	Air	Vacuum	Air	Vacuum	Air
Carbon Dioxide	0.3	0.3	0.1	0.1	27.9	27.7	0.1	0.5
Oxygen	0.1	0.1	0.1	0.1	0.3	0.4	20.3	18.6
Nitrogen	1.5	1.5	0.8	0.8	8.5	8.1	78.4	79.4
Water	0.5	3.8	0.2	1.1	0.5	2.1	0.3	0.3
Hydrogen	95.7	93.6	98.7	94.6	0.6	---	---	0.2
Silicon Tetrafluoride	---	---	---	---	59.9	57.5	---	---
Hydrogen Chloride	---	---	---	---	2.3	---	---	---
Ethylene	1.2	---	---	---	---	---	---	---
Acetone	0.3	---	---	3.2	---	4.3	---	---
Butene	0.1	0.6	---	---	---	---	---	---
Propene	0.3	---	0.1	---	---	---	---	---

\*Not corrected for residual air

Table IX

## Halogen Liberated During Irradiation

Package	Halogen	0 megarad	1 megarad	3 megarad	6 megarad
Fluorothane: Halogen found in 100 cc H <sub>2</sub> O in 4 x 6 bag	Fluorine ppm	0.1	0.6	1.1	1.1
Saran 6D: Halogen extracted from bags weighing 3.87 g.	Chlorine micrograms/gram	.008	0.016	0.052	0.104

Table I  
Effect of Radiation on Physical  
Properties of Plastic and Plastic Laminations

Package	Dose (Megarad)	Tensile Strength (lbs/inch)	Elongation (percent)	MVTR (gm/100 sq in/ 24 hrs/100 F - 90% RH)
Polymer Coated Cellophane <sup>19</sup>	0	28.5*	8*	0.70*
	1	28.0*	7*	0.78*
	5	25.5*	7*	0.86*
Polyethylene Coated Polyethylene Terephthalate <sup>20</sup> (Type D)	0	13.65	87.4	0.528
	1.2	13.17	81.9	0.505
	3.0	13.77	81.4	0.505
	6.0	13.27	82.6	0.562
Polyethylene (Medium Density) <sup>9</sup>	0	5.39	515	---
	1	5.01	490	---
	3	5.36	480	---
	6	5.48	490	---

\*Measured at 75° F - 81% RH

Table XI

## Effect of Radiation on Physical Properties of Papers

Package	Dose (Megarad)	Tensile Strength (lbs/inch)	Elongation (percent)	MVTR (gm/100 sq in/ 24 hrs/100°F - 90% RH)
Waxed Paper (Frozen Food Grade) <sup>21</sup>	0	9.3	17.6	1.31
	0.5	9.1	18.6	0.57
	1.0	8.9	20.4	0.63
	5.0	8.6	18.4	0.91
Waxed Kraft Paper <sup>21</sup>	0	19.0	25.6	0.62
	0.5	17.8	26.0	0.34
	1.0	17.7	25.0	0.33
	5.0	16.8	20.6	0.46
27# Heat Sealable Glassine <sup>22</sup>	0	---	---	0.438
	0.5	---	---	0.347
	5.0	---	---	0.414
47# War Laminated Glassine <sup>22</sup>	0	---	---	0.297
	0.5	---	---	0.279
	5.0	---	---	0.491

Table XII

Dye Penetration of Packaging Films  
After Rough Handling

Film	Undamaged	Creased TAPPI -T 465am 52	Galbo-flexured Flexures Before Failure
Saran 0.002 inch	0	0	200
Polyethylene, Low Density, 0.002 inch	0	0	500
Polyethylene Coated Polyester, Type M, 0.0025 inch	0	0	100
Polyethylene Coated Polyester, Type D, 0.003 inch	0	0	100

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V. STUDIES OF THE WHOLESOMENESS AND NUTRITIONAL ADEQUACY OF IRRADIATED FOODS

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|---|--|
| 1. Irradiated Food Studies                    | F. R. Blood<br>R. W. Engel<br>USAMENL<br>M. W. Hale<br>M. W. Hale<br>B. E. Kline<br>P. S. Larson<br>S. A. Miller<br>P. M. Newberne<br>E. F. Reber<br>L. E. Richardson<br>I. J. Tinsley |
| 2. Reproduction Studies                       | J. K. Loosli   |
| 3. Enzyme and Animal Tissue Studies           | M. Brin<br>K. J. Monty   |
| 4. Carcinogenicity Studies                    | E. E. Kline<br>S. W. Thompson  |
| 5. Hemorrhagic Syndrome Studies               | E. A. Doisy<br>B. C. Johnson<br>S. J. Mellette   |
| 6. Pathology Studies                          | J. F. Bone<br>R. H. Rigdon   |
| 7. Radioisotopes in Radiation-Processed Foods | G. R. Meneely  |

LONG TERM FEEDING OF IRRADIATED CHICKEN AND JAM  
TO DOGS, BEEF TO RATS AND DOGS, AND ORANGES AND PEACHES TO MONKEYS

Frank R. Blood and William J. Darby  
Vanderbilt University School of Medicine

I. STUDIES WITH DOGS RECEIVING IRRADIATED CHICKEN, JAM, AND BEEF  
( $2.79 \times 10^6$  and  $5.58 \times 10^6$  RADS.)

Diets were prepared by incorporating the control and irradiated foods with Purina Laboratory Meal as 35% of the solid content. All animals appeared to relish the three (3) types of diets fed.

Growth was erratic with all animals receiving the jam diet and in male dogs receiving irradiated beef but no correlation could be made between the type of diet and the growth response. Food consumption and feed efficiency were similar for animals on all three foods.

Urine analyses for animals receiving beef and chicken were negative except for a slight glycosuria in three of the four animals receiving irradiated chicken at the X-level and one on the 2-X level diet. All animals receiving the irradiated jam diets showed glycosuria on most tests. This was attributed to the high carbohydrate content of the diet and was verified by quantitative determinations of the fasting blood sugar level; all animals approached a normal value after a 24 hour fasting period.

Hematologic values varied slightly. Eosinophilia developed in most of the dogs at about the same period (24th to 36th week on experiment). Since this condition appeared in animals on all types

of treatment it is not felt that it was related to any specific diet.

Reproduction was very poor in all animals. Only nine of the eighteen females became pregnant. None of the control bitches on chicken and jam and only one animal on beef conceived after multiple attempts at breeding. Since our animals were maintained in metabolism cages throughout the entire experiment it was concluded that housing rather than the type of diet was responsible for the poor breeding performance.

II. STUDIES WITH MONKEYS RECEIVING IRRADIATED PEACHES ( $2.79 \times 10^6$  and  $5.58 \times 10^6$  RADS.) and WHOLE and PEELED ORANGES (150,000 and 300,000 REPS.)

Rhesus monkeys obtained from India and Pakistan were used on this project. It was not possible to procure animals of the same age and size because of the great demand for monkeys for preparation for Salk vaccine at the time this project was initiated.

Growth data were difficult to interpret because of the variation in food habits of the individual animals. Continual care was necessary in order to maintain adequate food intake and many techniques were used in an attempt to entice the animals to eat their diets. Also, since the monkey is susceptible to many infections and diseases, it is important to take great care in the selection and supervision of animal caretakers. It was generally concluded that the growth was satisfactory considering all variables present.

A rather severe eosinophilia developed in most of the monkeys at about the 24th week. This responded to treatment with chloromycetin and terramycin. All animals appeared to reflect minor changes in the

blood picture, but no consistent differences between groups could be attributed to the type of treatment.

Because of reports that monkeys receiving orange peel developed an increased ocular tension, intraocular pressure was measured on all animals receiving oranges. There was no detectable increase in the intraocular pressure measurement in animals receiving whole oranges but there was considerable variation from measurement to measurement. It is not believed that our data support the suggestion that a component of orange peel acts to produce glaucoma in the monkey.

### III. RATS RECEIVING IRRADIATED BEEF ( $2.79 \times 10^6$ and $5.58 \times 10^6$ RADS.)

The study was a single food design with three (3) replicates. These animals were fed diets containing irradiated beef as 35% of the solids incorporated with Purina Laboratory Meal. All animals appeared to grow satisfactorily and there was no undue mortality in any of the groups. Hematologic studies were normal throughout and no hemorrhagic syndrome was observed.

There was no evidence that reproduction was impaired due to feeding of the irradiated foods. Most of the male and female rats were fertile and could produce more than one litter of young. Animals in replicate 1 had the poorest fertility record but this was not associated with the diet received.

#### SUMMARY

It can be concluded from studies exclusive of histopathology that no adverse effects were demonstrated due to the feeding of any of the diets in the three species of animals. This conclusion was based on growth, hematology, food efficiency, reproduction, mortality and gross pathology.

## DETERMINATION OF NUTRITIVE VALUE OF IRRADIATED PROTEINS

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Virginia Polytechnic Institute

Previous work at Virginia Polytechnic Institute has shown that feeding either shrimp or carrots, irradiated or non-irradiated, had no deleterious effects on dogs. The rations contained 35% (food solids) of the test materials. Weight gain and feed efficiency were used to determine the effect of the irradiated and non-irradiated feeds.

Current work has been based on the examination of blood and serum from adult and young dogs fed a ration containing frozen shrimp, and shrimp irradiated with 3 million rep and 6 million rep.

Blood chemistry, hematologic examination and electrophoretic examinations were done on blood and serum from each dog. A base-line or norm for each dog was established over a 6 months period so animals could be selected for experiments. Hematologic examination revealed no significant difference in the red cell count, white cell count, differential count, hemoglobin or hematocrit. No significant differences were noted in serum non-protein nitrogen, chlorides, albumin, globulin, gamma globulin, A/G ratio, or total protein.

Serum electrophoresis patterns showed no significant difference between dogs on experimental rations or from the individual dogs norm.

All dogs were immunized with rabbit serum in order to study their response to a multiple antigen system. Anti-rabbit dog serum from each animal was examined using the double diffusion techniques of Oakley and Fuithrope, and Ouchterlony. Preliminary results have not

revealed any significant differences in the dogs' response to the antigen.

At the termination of the experiment all dogs were killed and necropsied. Gross lesions were not observed in any of the animals. Preliminary histopathologic examination of the organ tissues of all dogs have not revealed lesions that might be attributed to the irradiated food.

More recent work of the same nature is being done with laboratory animals. Serum electrophoresis patterns, blood chemistry and hematologic studies of mice, hamsters and rabbits fed a diet containing 2 levels of irradiated carrots (2.79 rad and 5.58 rad) as well as non-irradiated carrots have not shown any significant differences between groups or between their previously established norm.

Hamsters fed the above diets will be challenged with a virulent strain of Leptospira pomona to determine any differences in their susceptibility to infection.

SUMMARY OF RESULTS OF PARTICIPATION IN THE  
QUARTERMASTER FOOD IRRADIATION STERILIZATION PROGRAM  
(1955 to present date)

The U. S. Army Medical Research and Nutrition Laboratory  
Fitzsimons General Hospital

This laboratory is the representative of the Office of The Surgeon General for the contracts let by that office.

The major endeavor of this laboratory in the irradiated food program has been to determine the possible toxicity of irradiated foods and also their nutritional value. The program has consisted of two major phases: (1) A series of short-term (8 weeks) subacute toxicity studies and (2) long-term chronic toxicity study of four-generations' duration. Additional studies have been pursued involving metabolizable energy, enzyme systems, effect of irradiation on milk protein and the effect of irradiation on vitamin K.

Subacute Toxicity

Toxicological studies on 14 foods (ground beef, spinach, cereal bar, fresh ham, peaches, powdered milk, haddock fillets, bacon, beets, green beans, turkey, whole kernel corn, strawberries and bread) have been completed and each food found to be nontoxic. Several nutritional abnormalities have shown up such as the oxalic acid problem in spinach and the bio-availability of vitamin K in beef and pork. However, these are not toxicity problems, but nutritional problems which can be corrected.

### Chronic Toxicity

Four generations of rats were fed a composite diet of nine foods: ground beef, cereal bar, fresh ham, peaches, powdered milk, haddock fillets, bacon, beets and green beans. This diet was supplemented with both vitamins and minerals. The food irradiation level was 6 million rads with comparable control foods in addition. Growth, breeding, lactation, metabolizable energy (as weanlings and adults), enzyme levels and longevity were recorded. No toxic effects were observed in this study. The metabolizable energy data suggested irradiated foods were more readily metabolized. Liver cytochrome oxidase enzyme activity was increased in the irradiated group, indicating a possible interference in fat metabolism. Growth, breeding, lactation, longevity and pathology results appear to be statistically the same as the control animals.

### Enzyme Systems

Liver and serum enzymes systems have been investigated as a possible indicator of nutritional or toxicological effects of irradiated foods on the biological system. Of the systems studied (succinic dehydrogenase, liver cytochrome oxidase, liver xanthine oxidase and erythrocyte transketolase), liver cytochrome oxidase is the one most affected by irradiated foods.

A higher value in liver cytochrome oxidase is seen when the food fed has been stored or irradiated. Although results indicate that the liver cytochrome oxidase activity is increased in rats fed a vitamin K-deficient diet (Johnson-type), a direct relationship of enzyme activity to vitamin K intake does not appear to be evident. A similar conclusion

is indicated for liver succinic dehydrogenase. Other factors in the diet appear to play a greater role in cytochrome oxidase activity than does vitamin K. Erythrocyte transketolase activity in the rat is found to be related to the dietary intake of thiamine. The addition of sorbitol or the use of high levels of fat in the diet retarded the drop in RBC transketolase activity that resulted from the feeding of a cooked irradiated or nonirradiated 15-item composite diet found to be deficient in thiamine.

#### Alteration of Milk Proteins

Skim milk protein alteration by gamma and ultraviolet irradiation results in decreased anaphylactic response in milk-sensitized guinea pigs. Gamma irradiation results in dosage-related increases in viscosity and in sulfhydryl and disulfide content. Whole and skim milk have been studied after direct irradiation and after the irradiation-distillation method. The irradiation-distillation technique seems to result in less protein alteration as exhibited by allergenicity and electrophoretic studies, but results in a greater loss of methionine than the direct irradiation technique. The degree of protein alteration is dependent upon the concentration of the milk, the butterfat content and the method of irradiation.

#### Vitamin K Metabolism

It was observed at Dr. B. C. Johnson's laboratory that a vitamin K deficiency occurred when irradiated beef was fed to rats which were not supplemented with vitamin K. At this laboratory, it was determined that the vitamin K requirement for the rat, when fed an irradiated beef diet,

is 4  $\mu$ g vitamin K<sub>3</sub>, 5  $\mu$ g vitamin K<sub>1</sub> and 8  $\mu$ g Synkayvite (vitamin K<sub>3</sub> diphosphate), based on normal prothrombin values. It is interesting to note that the requirement for vitamin K<sub>3</sub> can be reduced 50% by the addition of bile to the irradiated beef diet.

Pork was also found to produce a vitamin K deficiency. It, however, is more severe than that produced by the beef and is prevalent in the animals fed the nonirradiated diet. A 50-100% increase in the disease may be attained by cooking and/or irradiating the meat.

This laboratory is currently interested in studying the utilization of vitamin K by using a radioactive-labeled compound and the development of a microbiological assay for vitamin K.

LONG TERM (CHRONIC TOXICITY) DOG FEEDING STUDIES OF IRRADIATED FOODS

A. CABBAGE

M. W. Hale, W. F. Schroeder, D. Sikes  
Animal Disease Department  
Georgia Coastal Plain Experiment Station

The effects of feeding a partially irradiated diet to dogs for a period of two years have been studied. The diet contained cabbage at the level of 20 per cent on a dry weight basis. One group of animals received the control, unirradiated cabbage which was maintained in a chilled condition prior to feeding; a second group received cabbage irradiated at  $2.79 \times 10^5$  rads and a third group received cabbage irradiated at  $5.58 \times 10^5$  rads. Irradiated and control cabbage was stored at  $4^{\circ}$  C for periods of three to four weeks prior to feeding. Data presented for these animals indicate no difference due to treatment with respect to growth, weight maintenance, hemoglobin, packed cell volume, white blood cell counts, reproduction and lactation. At the termination of the experiment no pathologic changes were found that could be attributed to the feeding of irradiated cabbage. (M. W. Hale, W. F. Schroeder and D. Sikes: GROWTH, REPRODUCTION, MORTALITY AND PATHOLOGIC CHANGES IN DOGS FED GAMMA-IRRADIATED CABBAGE FOR TWO YEARS).

LONG TERM (CHRONIC TOXICITY) DOG FEEDING STUDIES OF IRRADIATED FOODS  
B. BACON

M. W. Hale, W. F. Schroeder, D. Sikes  
Animal Disease Department  
Georgia Coastal Plain Experiment Station

The effects of feeding a partially irradiated diet to dogs for a period of two years have been studied. The diet contained bacon at the level of 35 per cent on a dry weight basis. One group of animals received the control, unirradiated bacon which was maintained in a frozen condition prior to cooking; a second group received bacon irradiated at  $2.79 \times 10^6$  rads and a third group received bacon irradiated at  $5.58 \times 10^6$  rads. Irradiated bacon was stored at room temperature ( $10^\circ$  to  $33^\circ$  C) for periods of six months to one year prior to feeding. Data presented for these animals indicate no difference due to treatment with respect to growth, weight maintenance, hemoglobin, packed cell volume, white blood cell counts, reproduction and lactation. At the termination of the experiment no pathologic changes were found that could be attributed to the feeding of irradiated bacon. (M. W. Hale, W. F. Schroeder and D. Sikes: GROWTH, REPRODUCTION, MORTALITY AND PATHOLOGIC CHANGES IN DOGS FED GAMMA-IRRADIATED BACON FOR TWO YEARS.)

THE LONG TERM FEEDING OF IRRADIATED POTATOES

B. E. Kline  
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White potatoes, irradiated with 0, 7500, or 1500 rad of gamma rays were fed to groups of 26 male and 25 female Sprague Dawley rats (litter mate distribution) through four generations.

The potatoes were steamed and mixed into a synthetic basal to provide 35% (wet wt.) of the diet.

The growth, reproduction, blood picture and pathology, including cancer incidence, was practically identical in all groups.

Survival was very good, amounting to 85, 83 and 83% at 18 months and 37, 46 and 50% at 24 months. No periarteritis was seen in any of the rats and about 20% of them exhibited respiratory disease.

We conclude that potatoes irradiated at these levels were not toxic to rats.

EFFECTS OF ADDING GAMMA-IRRADIATED GREEN BEANS OR  
FRUIT COMPOTE TO THE DIET OF DOGS

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Irradiated (3 and 6 megarep) and nonirradiated green beans and fruit compote have been added to the diet of pure-bred beagle dogs in amount equal to 35% of the dry solids fed for a period of two years. No effects attributable to irradiated food were apparent as regards growth, food efficiency, reproduction, hematology, or histopathology. A possible exception of unknown biologic significance was a trend toward increased spleen weights in animals receiving the irradiated food.

THE WHOLESOMENESS OF IRRADIATED DRIED WHOLE EGGS

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Research was initiated early in 1954 in our laboratories to determine the wholesomeness and potential toxicity of irradiation sterilized dried whole eggs. These studies were carried out in 3 phases. The first phase was concerned with the qualitative and quantitative changes in a number of essential nutrients of dried whole eggs treated with high energy electrons. In the second phase, the effect of feeding rations containing concentrations of irradiated dried whole eggs of 20 - 80 percent for 90 days to rats was studied. The third and final phase of this investigation consisted of a chronic study which was in progress for 2 years and 2 generations in the rat and 5 years and 1 generation in the dog. During this third part of the investigation, the irradiated eggs were included as 20 per cent of the rations.

The radiation treatment consisted of doses ranging from 1 - 3 megarep of high energy electrons. The eggs were packed in polyethylene bags and stored at temperatures ranging from room to -40° F. A portion of the eggs treated with doses of 3 megarep were packaged under low oxygen tension in cans.

No significant changes in nitrogen and fat concentrations in the eggs were noted upon irradiation. On the other hand, significant

\*Deceased

reductions in vitamin A and vitamin E were observed following irradiation treatment. The greatest loss in vitamin A was encountered when the eggs were packed under low oxygen tension prior to irradiation.

During the 90 day subacute study, no significant differences were observed in food utilization, caloric efficiency, hematology, and histopathology between animals fed irradiated and control eggs. However, a slight reduction in the growth of animals fed 80 per cent of the ration as eggs irradiated to 3 megarep was observed.

In addition to the experimental parameters outlined for the subacute study, the chronic rat study also included measurements of protein excretion, wound healing, and reproductive performance. In general, no significant differences between the various experimental groups in any of the experimental determinations were observed. In addition, tissues taken at periodic intervals throughout the test revealed no evidence of any pathological process which could be attributed to the feeding of irradiated eggs.

Similar results were obtained during the chronic study with dogs. However, in terms of reproductive performance, some difficulty was encountered. These abnormalities appeared in 2 forms, the first, a complete lack of the observable signs of estrus while in the second the young died after parturition. No correlation between the irradiation treatment of the eggs and these difficulties could be determined.

Since eggs constituted a significant portion of the diet, biotin supplementation was attempted in half of the animals of each group. Following the initiation of this program, 3 litters, all derived from

animals fed irradiated eggs, were satisfactorily whelped and raised to weaning. At the conclusion of the study, tissues were taken from all animals for histopathological examination. No evidence of any abnormality attributable to the feeding of irradiated eggs could be determined.

The results of these studies, therefore, indicate no significant effect of feeding relatively large quantities of irradiated dried whole eggs to animals for extended periods of time.

## IRRADIATED FOOD STUDIES

Paul M. Newberne  
Auburn University

The early work under this contract was concerned with the radiation effects on water soluble vitamins in raw beef. Three methods of assay were used including biological, microbiological and chemical. The values obtained by use of the three methods compared favorably with each other. Assay showed that approximately 3 megarep irradiation of raw beef resulted in destruction of about 60% of thiamine, 10% of riboflavin, and 25% of pyriodoxine. Niacin, choline, folacin, tryptophan and inositol were detectably decreased by ionizing irradiation treatment.

Long term studies were conducted to determine possible harmful effects of irradiating cod fish and sweet potatoes. Rats and dogs were the test animals. Two levels of irradiation, 2.79 and 5.58 Megarad, were used on each of the two foods. Latin square design experiments were set up using Sprague-Dawley and AES strains of rats and purebred Beagle pups. Later, due to the high incidence of respiratory disease in Sprague-Dawley rats, two additional groups of the AES strain of rats were included in the experiment. The original plan called for maintaining the animals on experiment for two years if they survived. Some of each group were selected for breeding and offspring were maintained through the 4th generation. Dogs were treated in a similar manner except that only a few of the bitches were bred and their offspring were not maintained on test. Studies included growth rate, reproduction and lactation, hematological data, longevity, and histopathology of tissues.

RATS:

Weight gains, food consumption and efficiency were about the same for animals receiving irradiated food as those consuming non-irradiated food. Breeding performance was poor for all groups in each generation. Many of the young born did not survive apparently due to poor lactation in the dam. Hemoglobin, red and white blood cell counts were generally within normal range for all generations in both groups. No differences were detected between those receiving irradiated food and those receiving non-irradiated food. However, white cell counts in all animals tended to be in the upper range of normal and this could usually be correlated with the chronic respiratory disease found in high incidence among all Sprague-Dawley animals. Survival time was about the same in all groups and no consistent difference was observed between irradiated and non-irradiated groups. In general, survival time was markedly less than had been anticipated due largely to respiratory disease and only a small number of animals remained on experiment for two years. Histopathologic studies showed no changes of significance in either group. The most striking observation was the low incidence of tumors and the high incidence of chronic respiratory disease.

DOGS:

The experiments with dogs showed results similar to those observed in rats. Initially the work was hampered by an outbreak of distemper

with a few losses that required replacements. The original protocol did not specify breeding performance but a later memorandum asked for breeding trials.

Only four litters of pups were obtained, however, due to the apparent failure of bitches to come into heat. An individual litter was obtained from the control group and each level of irradiation except for one treatment (2.79 Mrad irradiated sweet potatoes).

Growth data, hematological studies, longevity and histopathologic examination of tissues revealed no differences attributable to feeding irradiated food. The outstanding lesion histologically was a form of cystic filamentous degeneration in the brain of several animals that was of questionable origin but not related to food irradiation since the changes were observed in animals from irradiated and non-irradiated food groups.

TO DETERMINE THE EFFECT OF IRRADIATION UPON THE WHOLESOMENESS OF FOOD

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Wholesomeness Test of Irradiated Beef

A final report has been made. No gross abnormalities or ill effects were observed due to feeding irradiated beef to dogs for two years. However, there was a significant decrease in food consumption during the 24-week growth period in dogs fed the 5.58 megarad radiated beef, but no significant differences were noted in efficiency of feed conversion or weight gains. It was concluded that feeding irradiated beef to dogs had no significant effect on the growth, feed conversion, fertility or lactation as compared to dogs fed nonirradiated beef; irradiated beef had no apparent hematological effects. Histopathological examination of tissues revealed no consistent lesions that could be associated with the feeding program.

Wholesomeness Test of Irradiated Flour

A final report has been made. Groups of 2 male and 2 female Beagles were fed a complete ration in which 35% of the total solids was nonirradiated flour (FO) or flour irradiated with 37,200 (FX) or 74,400 (F2X) rads. The dogs were fed the rations during a 104-week period. There was no statistically significant difference in the initial weight, weight gained or feed consumed between the dogs fed irradiated flour and those fed nonirradiated flour. All the females and males were fertile. The bitches whelped 21, 13 and 16 pups and weaned 9, 1 and 5 pups in the

FO, FX and F2X groups respectively. Two bitches, one in treatment FO and one in FX, had one litter during the experimental period. The number of pups whelped appears to be approximately what would be expected of these animals. The percentage of pups weaned of those born was quite low in all treatments. The low weaning rate appears to be related not to radiation treatment but to whelping difficulties and accidents. The dogs had a tendency to obesity and the birth weights of the pups were high. The males in this experiment were large for the Beagle breed weighing 13 to 19 kg.

It is concluded that feeding irradiated flour to dogs had no significant detrimental effect on growth, feed conversion, fertility or lactation as compared to dogs fed nonirradiated flour. It is concluded that irradiated flour had no apparent effect on the blood level of hemoglobin, red blood cell, white blood cell or differential white blood cell counts as compared to the blood values of the dogs fed non-irradiated flour. Thyroid lesions increased with increasing level of irradiation, but the data are too few to achieve statistical significance.

#### The Hemorrhagic Syndrome in Male Rats, Cats and Dogs

The Sprague-Dawley strain of rat was more susceptible to the hemorrhagic disease than was the Holtzman strain of rat.

A ration containing radiated beef (2.79 megarads) which was hemorrhagic for male rats had no such potential when fed to male cats and male dogs for 40 weeks.

The occurrence of the disease in male rats was reduced by castration. The occurrence of the disease in castrated male rats was

increased by the administration of testosterone. The amount of vitamin K required by the castrated male rat to prevent prolonged prothrombin time and death due to hemorrhage increased directly as the level of testosterone was increased. When 2 gamma of vitamin K were given and testosterone was administered at the rate of 0, 0.25, 0.5 or 1.0 mg. the percentage deaths were 0, 20, 66 and 100, respectively.

Nonirradiated beef contains 33 gamma of K, per 100 gm. (on dry basis) (B. C. Johnson, Report 17). Irradiation of beef causes a 50% (B. C. Johnson, Report 17) or 85% (Desrosier, Radiation Technology) loss of vitamin K.

Tsien and Johnson (J. Nutrition 69, 45, 1959) reported 2.93, 2.45 and 2.16 gm. L-methionine per 100 gm. of protein in nonirradiated, 2.79 and 5.58 megarad radiated beef, respectively. Thomas and Calloway (QMFCIAF Report N R 2-61) found 2.37, 2.64 and 2.22 gm. of L-methionine per 100 gm. of protein in nonirradiated, irradiated (4.8 megarad) enzyme inactivated and irradiated precooked, respectively.

Only the work of Tsien and Johnson was available when we started an investigation of the effects of adding methionine to the hemorrhagic rations. Weanling and adult male Sprague-Dawley rats were fed rations containing nonirradiated or irradiated (5.58 megarad) beef. None of the weanling rats fed nonirradiated beef died. When the ration containing irradiated beef supplemented with 0, 0.17, 1.0, 1.5 and 2% DL-methionine was fed 89, 100, 60, 7 and 0% of the weanling rats died, respectively. When the ration containing 0, 0.17, 1.0, 1.5 and 2% DL-methionine was fed to adult rats 90, 80, 50, 0 and 0% died, respectively. In the absence of dietary vitamin K 2% DL-methionine protected

the weanling and 1.5% DL-methionine protected the adult against death due to hemorrhages.

The relationship of methionine to vitamin K was explored. Vitamin K (menadione) was administered orally at levels of 0, 1, 2, 3 and 4 gamma 3 times a week. The intake of vitamin K by rats fed the 3 or 4 gamma level and irradiated beef was equivalent to that taken in the diet by rats fed nonirradiated beef when the loss of vitamin K due to irradiation was considered to be 50 or 70%, respectively. At the end of an 8 weeks experimental period the following combinations protected against hemorrhages and death: 2 gamma and 1% DL-methionine, 3 gamma and zero DL-methionine. There appears to be a direct sparing effect of methionine on the vitamin K required to prevent hemorrhages and prolonged prothrombin times. Adding methionine (0.17% DL) and vitamin K (3 or 4 gamma level) to the ration containing irradiated beef would bring the levels of these two nutrients near to their levels in non-irradiated beef rations and in so doing prevents the occurrence of deaths due to hemorrhages. Nonirradiated beef contained 2.93 and irradiated beef + 0.17% DL-methionine contained 3.70 gm. methionine per 100 gm. of beef.

The relationship of methionine and testosterone to the incidence of the hemorrhagic disease was explored. Testosterone was administered subcutaneously to castrated male Sprague-Dawley rats. The percentage of deaths increased with increasing levels of testosterone. The effect of testosterone was counteracted by increasing the dietary level of DL-methionine.

the weanling and 1.5% DL-methionine protected the adult against death due to hemorrhages.

The relationship of methionine to vitamin K was explored. Vitamin K (menadione) was administered orally at levels of 0, 1, 2, 3 and 4 gamma 3 times a week. The intake of vitamin K by rats fed the 3 or 4 gamma level and irradiated beef was equivalent to that taken in the diet by rats fed nonirradiated beef when the loss of vitamin K due to irradiation was considered to be 50 or 70%, respectively. At the end of an 8 weeks experimental period the following combinations protected against hemorrhages and death: 2 gamma and 1% DL-methionine, 3 gamma and zero DL-methionine. There appears to be a direct sparing effect of methionine on the vitamin K required to prevent hemorrhages and prolonged prothrombin times. Adding methionine (0.17% DL) and vitamin K (3 or 4 gamma level) to the ration containing irradiated beef would bring the levels of these two nutrients near to their levels in non-irradiated beef rations and in so doing prevents the occurrence of deaths due to hemorrhages. Nonirradiated beef contained 2.53 and irradiated beef + 0.17% DL-methionine contained 3.70 gm. methionine per 100 gm. of beef.

The relationship of methionine and testosterone to the incidence of the hemorrhagic disease was explored. Testosterone was administered subcutaneously to castrated male Sprague-Dawley rats. The percentage of deaths increased with increasing levels of testosterone. The effect of testosterone was counteracted by increasing the dietary level of DL-methionine.

increased by the administration of testosterone. The amount of vitamin K required by the castrated male rat to prevent prolonged prothrombin time and death due to hemorrhage increased directly as the level of testosterone was increased. When 2 gamma of vitamin K were given and testosterone was administered at the rate of 0, 0.25, 0.5 or 1.0 mg. the percentage deaths were 0, 20, 66 and 100, respectively.

Nonirradiated beef contains 33 gamma of K, per 100 gm. (on dry basis) (B. C. Johnson, Report 17). Irradiation of beef causes a 50% (B. C. Johnson, Report 17) or 85% (Desrosier, Radiation Technology) loss of vitamin K.

Tsien and Johnson (J. Nutrition 69, 45, 1959) reported 2.93, 2.45 and 2.16 gm. L-methionine per 100 gm. of protein in nonirradiated, 2.79 and 5.58 megarad radiated beef, respectively. Thomas and Calloway (QMFCIAF Report N R 2-61) found 2.37, 2.64 and 2.22 gm. of L-methionine per 100 gm. of protein in nonirradiated, irradiated (4.8 megarad) enzyme inactivated and irradiated precooked, respectively.

Only the work of Tsien and Johnson was available when we started an investigation of the effects of adding methionine to the hemorrhagic rations. Weanling and adult male Sprague-Dawley rats were fed rations containing nonirradiated or irradiated (5.58 megarad) beef. None of the weanling rats fed nonirradiated beef died. When the ration containing irradiated beef supplemented with 0, 0.17, 1.0, 1.5 and 2% DL-methionine was fed 89, 100, 60, 7 and 0% of the weanling rats died, respectively. When the ration containing 0, 0.17, 1.0, 1.5 and 2% DL-methionine was fed to adult rats 90, 80, 50, 0 and 0% died, respectively. In the absence of dietary vitamin K 2% DL-methionine protected

## NUTRITIONAL VALUE OF IRRADIATED FOODS

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Rate of growth and reproductive performance of four generations of rats receiving an irradiated synthetic diet were essentially equal to those of animals receiving the nonirradiated diet. Four generations of rats received nine different combinations of green beans and boned chicken, each irradiated at dose levels of zero, 2.79 and 5.58 megarads. Irradiated foods were stored at room temperature. Control foods were stored at  $-20^{\circ}$  C. No significant differences in gain in weight at 12 weeks, gross or histopathology abnormalities occurred in the parent  $F_1$  and  $F_2$  generations which could be attributed to the irradiated food.

Addition of 10% irradiated animal fatty tissue to the diet resulted in poor growth and high mortality in chicks when the diets were stored for 1 to 3 weeks before being fed. Addition of an antioxidant (BHT) to the fatty tissue before irradiation or to the diet at the time it was mixed resulted in normal growth and low mortality. Diets containing irradiated masola (corn oil) and soybean oil could be stored for 1 to 3 weeks with minor loss in nutritional value.

Vitamin K activity of foods frozen, canned by heat treatment and irradiated with 2.79 and 5.58 megarads of gamma rays was not affected by the method of preservation.

The vitamin B<sub>6</sub> activity of foods was changed by the method of preservation and by the length of the storage periods. Conventional heat treatment and irradiation consistently decreased the vitamin B<sub>6</sub> activity of the foods. In general, the amount of activity retained in the irradiated foods was essentially the same or greater than that in the heat treated foods. Four of 6 foods decreased and 2 increased in vitamin B<sub>6</sub> activity during a storage period of 15 months. This difference may be due to the form of vitamin B<sub>6</sub> present.

Male rats of the Holtzman strain receiving irradiated beef, ether extracted irradiated beef or irradiated soybean protein (ADM assay protein C-1) developed a high incidence of the hemorrhagic syndrome. Some hemorrhages developed in rats receiving nonirradiated soybean protein, but the incidence was lower than in those receiving irradiated protein. Increasing the level of soybean protein increased the incidence of the hemorrhages. Hemorrhages were also higher when the diet contained 60,000 IU of vitamin A than when it contained 30,000 IU/kg. Addition of 4.0% of a mixture of 13 pure amino acids decreased the incidence and severity of the hemorrhages. The diet of mothers had a marked effect on the susceptibility of the offspring to develop hemorrhages. The diet of the breeding colony may explain some of the difference which has been attributed to strain of rats used in the hemorrhage studies.

## WHOLESOMENESS OF IRRADIATION STERILIZED FOODS

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Oregon State University

The wholesomeness of irradiation processed pork, peaches, jam, carrot, and flour has been evaluated in long term feeding experiments using rats as the experimental animal. In addition, tissue enzyme studies have been carried out.

### 1. Growth, Breeding and Longevity.

No statistically significant treatment effects were observed in the jam, peach and flour experiments. The following statistically significant effects have been observed:

(a) Pork Ration. Significant decrease in weaning efficiency of animals raised on the 2.79 megarad pork ration. This effect was due to a low figure in the parent generation. Since this difference was not apparent in subsequent generations, it is not considered to have much bearing on the evaluation of the overall experiment.

(b) Carrot Ration. The number of litters produced for a given number of breeding opportunities was lower with animals on the control carrot rations. This was due, not to an increase in the number of sterile females but to the fact that a smaller number of females on the control carrot ration produced two litters.

(c) Carrot Ration. Animals raised on the control carrot ration showed a higher rate of growth than those on the irradiated carrot rations. This effect is more pronounced in the male animals and also \*(deceased)

there is no difference in growth rate in animals raised on the two irradiated carrot rations.

## 2. Tissue Enzyme and Miscellaneous Studies.

(a) Carotene utilization in irradiated carrot rations. The level of liver vitamin A in rats raised on control carrot rations is considerably higher than that of rats raised on the 5.56 megarad rations. This effect may be explained in part by a difference in the isomeric composition of the carotenes of the two rations.

(b) Tissue enzyme effects. In comparing animals on the irradiated rations with those on the corresponding control ration the following effects were observed:

decrease in fructose oxidation, heart homogenates--jam ration

decrease in fatty acid oxidation, kidney mitochondria--pork ration

increase in DNP-ATP-ase, liver mitochondria--pork ration

increase in cytochrome oxidase liver mitochondria--pork ration

Experiments in the lab have not indicated any correlation between vitamin K or vitamin E supplements and cytochrome oxidase activity.

Differences have been observed in the reaction of liver homogenates from animals on the control and irradiated pork rations with 2-thiobarbituric acid. This effect could be explained by differences in the fatty acid composition of the livers of the two groups of animals. Studies of the fatty acid composition of liver lipides have been made but the results have not been summarized as yet.

COMPONENTS OF IONIZED IRRADIATED MEATS INJURIOUS TO REPRODUCTION

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Thirty-six registered pure bred beagles between 8 and 10 weeks of age were started on experiment soon after weaning, on September 19, 1960. The dogs were randomly assigned to the groups after having been divided into littermate pairs where possible. They were examined for physical abnormalities as well as for parasites. Since all dogs were born in our kennel, they were vaccinated between 2 to 15 weeks of age depending on the blood titer of the bitch at the time of whelping. Lederle's distemper and hepatitis-modified live virus vaccine was used. Weights are taken and physical examinations given weekly. Parasite and hematological examinations are made at regular intervals.

Electron beam irradiated beef has been used. Both the control and irradiated beef are being fed as 35% of the dry matter, the remaining 65% being made up of a commercial meal type ration. The irradiated beef is stored at ambient temperature while the control is held frozen until the day before it is used. Small volumes of tap water are added to facilitate thorough mixing of the total ration. The dogs are given vitamins orally at least two hours before or after feeding. Each dog receives 40,000 units of A, 4,000 units of D<sub>3</sub>, and 400 units of E weekly. Every dog at the start of the experiment received 100 calories per pound of body weight per day, but this amount has been decreased as the dog matured. The dogs went on a once daily

feeding schedule on November 7, 1960, approximately two months after the experiment started.

The growth curves of both dietary groups are normal and similar. The dogs on this experiment are growing and maturing faster than usual compared with our kennel normals. This is probably attributable to the superior nutritive value and high acceptability of this ration.

EFFECT OF FEEDING X-IRRADIATED FOODS ON ENZYME LEVELS IN BLOOD

Myron Brin\*

Food and Drug Research Laboratories, Inc.

1. Purpose: The evaluation of the nutritive value of four x-irradiated foods as measured by 5 blood enzyme systems in the rat.

2. Basis: It has been known that x-irradiation may destroy certain nutrients and sulfhydryl groups in foods. It was desirable to evaluate the biological significance of these phenomena as they may be reflected in the enzymes of a readily available biopsy tissue, namely blood.

3. Exploratory studies on enzyme assay methods.

a. Thiamine evaluation: A new hemolysate assay was developed for the transketolase enzyme in erythrocytes. Two measures of enzyme activity are used, namely, the disappearance of pentose, and the accumulation of hexose. The assay was highly sensitive to thiamine deficiency before growth was affected.<sup>1</sup>

b. Pyridoxine evaluation: It was demonstrated that serum glutamic-alanine and glutamic-aspartic transaminases were very sensitive to pyridoxine deficiency under controlled conditions. The effect was as effective in heavy, slow-growing rats as in younger, rapidly-growing animals.<sup>2</sup>

c. Three additional enzyme systems: erythrocyte (1) glycolysis, (2) aerobic oxidation of glucose with methylene blue, and (3) glutathione reductase. No further developmental work was required.

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4. Experimental Program:

(a) Pork-Thiamine.

Unirradiated pork, when incorporated into diets at a level of 35% on a dry weight basis, contained sufficient B<sub>1</sub> to maintain normal growth and erythrocyte transketolase. X-irradiated pork, with no B<sub>1</sub> added, resulted in depressed growth (53 and 63%) and depressed transketolase (83 and 94%) for the 1x and 2x levels respectively after a 4 week feeding period. Treatment of the rats with B<sub>1</sub> resulted in resumed growth and increased transketolase.<sup>3</sup>

(b) Pork-Pyridoxine.

No differences in growth or aspartic transaminase were noted when unirradiated or x-irradiated pork (1x and 2x levels) were fed to rats at a level of 35% of the diet on a dry weight basis, over a 12 week period. However, the alanine enzyme was depressed on unirradiated pork with no B<sub>6</sub> added, and on x-irradiated pork with no B<sub>6</sub> added, increasingly in that order.<sup>4</sup>

(c) Pork, bread, green beans, and shrimp and 5 blood enzyme systems.

Pork, green beans, and shrimp (2.79 and 5.58 megarad) and bread (25 and 50 kilorad) were fed to rats at a level of 35% of the diet on a dry weight basis, for a period of 12 weeks. Bread, green beans and shrimp were also fed at a level of 80% of the diet. All diets were adequately supplemented with vitamins and minerals, and in the case of green beans, with added protein. Growth, appearance and behavior were normal for the 12 wk. period. Blood enzyme levels of erythrocyte transketolase, serum transaminase, erythrocyte glycolysis, erythrocyte aerobic oxidation of glucose with methylene blue and erythrocyte

glutathione reductase were normal. No toxic signs were noted.<sup>5</sup>

5. Significance of Program:

a. When the irradiated foods were supplemented with vitamins, no toxic signs were noted, whether physical, physiological or chemical.

b. The use of erythrocyte transketolase and serum transaminase to evaluate adequacy in the rat for thiamine and pyridoxine, respectively, was supported.

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EFFECTS OF HIGH LEVELS OF IONIZING RADIATION ON ANIMAL TISSUES

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The efforts under this contract have been focused upon the question of whether the carbonyl compounds resulting from the exposure of meats to preservative doses of ionizing radiation constitute a health hazard upon chronic ingestion.

A joint study between our laboratory and Drs. Tappel and Groninger of the University of California (Davis) successfully elucidated the nature and the amounts of carbonyl compounds encountered in various meats after exposure to preservative doses of radiation. Three groups of compounds were recognized: long chain aliphatic carbonyl compounds, probably representing hydrolytic breakdown products of the class of phospholipids known as plasmalogens; aliphatic aldehydes and ketones having chain lengths of from 2 to 12 carbon atoms, typical of the products of peroxidative degradation of unsaturated fatty acids; and a group of keto acids, largely uncharacterized, probably resulting from the oxidative deamination of amino acids. The keto acids were presumed to be least likely to constitute a health hazard upon ingestion, and were not studied further. All subsequent efforts were concentrated upon the carbonyl compounds of lipid origin, which successfully survived both storage and cooking of the meats in which they were contained.

Studies with aliphatic carbonyl compounds have revealed the following. Such compounds act as inhibitors of pancreatic lipase and also of lipid absorption from the intestinal lumen. These two

phenomena probably have a common explanation in the fact that such carbonyl compounds, because they are surface active, modify the properties of interfaces between lipid and water phases.

The aldehydes found in irradiated meats can be readily metabolized by several metabolic pathways in mammalian tissues. For example, aldehyde dehydrogenase, a pyridine nucleotide - dependent system, will readily catalyze their conversion to fatty acids. The probable metabolic fate of ketones is not clear.

Aliphatic aldehydes will inhibit plasmalogen synthesis as studied in vitro. Neither the nature nor the significance of the observation has been clarified.

The forced feeding of large amounts of aliphatic aldehydes and ketones to rats daily for periods of 2 to 4 weeks produced no recognized adverse effects. Attempts to find evidence for accumulation of the fed carbonyl compounds within the carcass were unsuccessful, even though the analytical techniques were of a high order of sensitivity. Only female animals were employed. Within the scope of these experiments, no evidence was found to suggest any hazardous aspect to the ingestion of carbonyl compounds.

#### Publications

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## THE POSSIBLE CARCINOGENICITY OF IRRADIATED FOODS

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Fresh pork brain and egg, commercial sterol concentrates high in cholesterol and ergosterol, a mixture of vegetable oils, a mixture of ground meat-fish-cheese-milk powder, and lard were subjected to beta or gamma irradiation and tested in rats and mice for the presence of a cancer producing substance.

The irradiation levels ranged from approximately  $10^5$  to approximately  $10^7$  rad. The nonirradiated materials were tested as controls.

The lard was injected and the other materials were fed to a total of 200 male and 200 female rats.

The lard was injected, the sterols and vegetable oils were fed, "painted" and injected, the brain and egg was fed and an alcohol, ether soluble extract of the brain and egg and of the meat-fish-cheese-milk was "painted" and injected into mice using a total of nearly 3700 mice.

The mice included random bred (Swiss), inbred (C3H) and hybrid (CAF1, BDF1).

No carcinogen could be demonstrated in the irradiated food preparations.

### A SUMMARY OF THE TUMOR INCIDENCE IN DIFFERENT STRAINS OF MICE

	No. of Mice	Tumors/100 mice
Swiss		
S-P	384	25
Sutter	1708	50
Controls	812	50.1 (Range 12 to 82)
Treated	896	50.0 (Range 8 to 72)
C3H Jax	672	31
CAF1 Jax	600	18
BDF1 Jax	400	4

\*presented paper

VARIATION IN TUMOR INCIDENCE IN DUPLICATE GROUPS OF SWISS MICE

(Number of tumors per 100 mice)

Controls	Group 1	48	Group 2	46
Treated		62		84
Controls	Duplicate	82	Duplicate	36
Treated		72		31

CARCINOGENICITY OF FOODS PRESERVED BY IRRADIATION

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Our work repeats and extends, at the request of The Surgeon General, the study conducted by Dr. Harry Monsen of the Department of Anatomy, University of Illinois, College of Medicine, on Department of the Army, Medical Research Contract #DA 49 007 MD 794. He reported the occurrence of a lesion which afflicted the left auricle of the heart of some mice of the Cb and Strong A strains. The incidence rate of this lesion incriminated the irradiated foods which were fed. The study being conducted at this laboratory is designed to further evaluate the reproducibility, pathogenesis and etiology of the left auricular lesions originally observed by Dr. Monsen. It is divided into 4 phases: (1) breeding of the required strains of mice; (2) repeat of Dr. Monsen's original protocol; (3) pathogenesis and (4) etiology.

The irradiated and the non-irradiated control diet consists of a composite of pork, chicken, milk (evaporated), white potatoes, and carrots (blanched), to which is added commercial vitamin and salt mixtures. A commercial laboratory rodent pellet diet is also being fed as a second control.

Progress:

Phase I - The breeding of the required number of mice has been completed and this phase of the project has been discontinued. All

\*presented paper

breeding stock, whose offspring were placed in another phase of the experiment, are being allowed to live out their lifespan. These mice will receive a complete necropsy at the time of their death and histopathological examination.

Phase II - All mice of both strains (1300 Cb and 1200 Strong A) have been placed on this phase. These mice are divided into three groups of paired littermates for each strain (425 Cb and 400 Strong A), and are being fed the composite irradiated diet, the non-irradiated control and the pellet diet respectively. Sacrifices are scheduled for 200, 300, 400, 500 and 600 days after being placed on the diets. The 200 day sacrifice commenced on 13 February 1961. To date the following numbers of mice have been sacrificed: 200 day, 414 Cb, 372 Strong A; 300 days, 132 Cb, 99 Strong A; 400, 500 and 600 day, none. All mice of this phase will be sacrificed by 31 May 1962.

Phase III - All mice of the Cb strain which were originally scheduled for this phase have been put on test. All 108 mice scheduled for this phase have been sacrificed in a total of 13 groups at 25 day intervals.

Phase IV - A total of 1600 mice have been placed on this phase. The mice are being fed a total of 20 diets, one ingredient of each diet being irradiated. Each of these five basic composite diets is then divided into four groups: cooked with vitamins, cooked without vitamins, uncooked with vitamins and uncooked without vitamins. Sacrifices are scheduled for 100, 200, 400 and 500 days after being placed on this diet. To date the following number of mice have been sacrificed: irradiated pork, 131 mice; irradiated chicken, 132 mice; irradiated milk,

131 mice; irradiated potatoes, 132 mice; irradiated carrots, 132 mice; a total of 658 mice. The sacrifices will be completed on 11 May 1962.

In October 1960, at the request of Colonel Hursh, a total of 84 mice of the Cb strain were placed on an irradiated diet similar to Phase II with the exception that the milk component was stored at 10° F rather than at room temperature. To date none of these mice have been sacrificed and there have been no deaths.

On 19 August 1960, at the request of Dr. Mosen, 72 mice of the Cb strain were placed on a milk diet fortified with vitamin mix. This milk was furnished by Dr. Mosen from his stocks. The animals of this group are divided as follows:

- Group 1 - 18 mice - uncooked irradiated milk,
- Group 2 - 18 mice - cooked irradiated milk,
- Group 3 - 18 mice - uncooked non-irradiated milk,
- Group 4 - 18 mice - cooked non-irradiated milk.

To date 66 mice have died of emaciation:

Deaths

- Group 1 - 15
- Group 2 - 15
- Group 3 - 18
- Group 4 - 18

Dr. Mosen is running a companion study.

At the suggestion of Major Joseph Berg (CMF&CI) a total of 72 mice of the Cb strain are being fed a diet similar to the Phase II diet. However, these mice are being fed from bean pots rather than disposable containers. Thirty six are being fed the irradiated diet, and 36 are

being fed the control diet. Eighteen mice of each group have been sacrificed to date.

Summary and Conclusions:

A total of 4,364 mice have been placed on the experiment and a total of 1,883 mice have died or been sacrificed to date. All tissue specimens (ca 37,400) from approximately 1,700 of these mice have been embedded in paraffin; however, histopathological preparation and examinations have been completed on less than 400 mice. Complete histopathological examinations have not been completed on enough mice from any phase to draw any statistical conclusions as to the occurrence of lesions in control vs experimental animals. Grossly recognizable dilatation and thrombosis of the left auricle and atrium, as described by Dr. Monsen, has not been observed. Six thrombi have been observed in microscopic sections of the heart. Three of these were in the left auricle, two in the right atrium and one in the coronary artery. All of these were present in mice subsisting on a 100% irradiated milk diet fortified with vitamins. Focal calcification of various tissues is a common finding in all phases of the experiment. The tissues most often affected are the heart (the myocardium of all four chambers), coronary arteries (media), skeletal muscle and kidney (urolithiosis). Occasionally lung, stomach (the mucosa) and subcutaneous tissues are affected. Myocardial calcification is frequently associated with hyalin degeneration. One tumor has been observed, a sebaceous gland adenoma. Carcinoma of the mammary gland is present in the older animals of the breeding colony.

## HEMORRHAGIC SYNDROME IN RATS

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Our contributions to the study of the hemorrhagic syndrome in rats fed irradiated beef have been given in five progress reports to the Office of The Surgeon General. Appropriate data from these reports have been mentioned in the bi-annual summary of progress issued by the Office and were also included in a paper presented by Mellette at the meeting of FASEB in Chicago in April 1960.

Briefly we have 1) contributed to the recognition of differences between strains of rats in response to the hemorrhagic syndrome; 2) contributed to the recognition of sex differences; 3) established that the previously known hemorrhagenicity of vitamin A at toxic levels is also operable at physiological levels, thus emphasizing the need for careful consideration of the level of dietary vitamin A in studies of vitamin K deficiency in the rat; and 4) devised a convenient assay for screening potentially hemorrhagenic diets.

The results of three phases of our work in progress were given in our report No. 5. A summary of these data was included in a paper presented by Doisy at the Federation meeting in April and will be published in December. Briefly these data are concerned with 1) the hemorrhagenicity of dietary lipids; 2) the role of dietary protein in nutritional hemorrhage; and 3) studies with  $^{14}\text{C}$  labeled vitamin  $\text{K}_1$  recently prepared in our Department.

In our studies on lipids, squalene was found to be markedly

hemorrhagic and its effect was further enhanced by partial oxidation with perphthalic acid. Fat from irradiated beef was no more hemorrhagic than that from fresh beef; however, both fats were more hemorrhagic after oxidation with perphthalic acid. Cottonseed oil was also more hemorrhagic after oxidation but purified samples of methyl oleate and methyl linoleate were ineffective even after oxidation.

In consequence to our studies on vitamin A we have examined its biologically interesting derivative, vitamin A acid. Dietary administrations of this compound showed that it was ten to fifty times more hemorrhagic than vitamin A acetate.

Our results of a quantitative comparison of several dietary proteins for their relative hemorrhagicity re-emphasized the need for consideration of the protein used in studies of vitamin K deficiency in the rat. Persistent differences in hemorrhagicity were observed even after thorough extraction of the proteins studied.

Vitamin K<sub>1</sub> labeled in the side chain with <sup>14</sup>C was prepared by William R. Michael in our department and biological studies are presently underway. Availability of the labeled vitamin will be helpful in several aspects of work on the nutritional hemorrhagic syndrome. In addition to absorption and metabolism studies examination of proteins and other foodstuffs as well as feces for their content and stability of the vitamin should be facilitated.

In evaluating our work, we attach considerable significance to the role of vitamin A in the induction of hemorrhage. The observed relation of small amounts of vitamins A and K in the maintenance of the coagulation process is of nutritional significance and on further examination

may provide the basis for a better understanding of the biological activity of both vitamins. The observed hemorrhagic effect of squalene and the enhancement of hemorrhagenicity by oxidation of certain dietary lipids is also of nutritional significance and is under further study. Our studies with various dietary proteins were undertaken in order to evaluate the relative hemorrhagenicity of protein prepared from fresh and irradiated beef. An understanding of the beef induced hemorrhages requires a quantitative knowledge of all operative factors. Our studies are directed toward this goal.

SUMMARY AND EVALUATION OF FINDINGS TO DATE  
FROM OUR PARTICIPATION IN THE  
FOOD IRRADIATION CONTRACT PROGRAM

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University of Illinois

Our studies on the wholesomeness and nutritive value of irradiation-sterilized foods have been concerned with the effect of irradiation on protein and amino acids, on energy, on long-term survival and reproduction, and on vitamin K.

Protein and Amino Acids

Our work on the biological value of the protein of irradiation-sterilized foods has established that in no case is serious damage done to the protein of the food studied, that is, milk, beef, corn, wheat, peas, lima beans, or isolated casein, by irradiation doses up to as high as 10 million rads. Some slight depression in biological value of milk (8%) and pea protein (8%) occurred at 6-million-rad irradiation.

Examination of the amino acid contents of the protein of peas and beans indicated that irradiation sterilization of peas and beans gave considerable destruction of lysine and arginine, although not sufficient to cause these two amino acids to become limiting. In both the milk and beef proteins, irradiation markedly destroyed glutamic acid, aspartic acid, serine, and glycine, but without influencing in any way the biological value of these proteins.

### Energy

Irradiation at a level of 3 million rad caused no loss in metabolizable energy value of carbohydrate, fat, or protein of synthetic rations.

### Long-Term Studies

In two separate long-term reproduction studies, using diets containing 35% beef irradiated at 3 million and at 6 million rad, completely satisfactory reproduction was obtained once vitamin K was included in the diet in experiment 1, and even better results were obtained when the animals were also kept in isolation and therefore free of respiratory disease. Histological examination of the tissues of rats from these experiments did not reveal any significant differences due to the irradiated food.

### Vitamin K

It was discovered that male rats fed diets containing irradiated beef and no supplemental vitamin K developed hemorrhages which could be prevented by vitamin K supplementation. Subsequent work had indicated that there is vitamin K destruction by irradiation in meat. However, meat is not a normal source of vitamin K, and this destruction of the small amount present certainly has no effect on the nutritive value in terms of normal diets.

### Fundamental Studies

In addition to the testing of the nutritive value of irradiation-sterilized foods, a large amount of fundamental information in the field

of nutrition has been obtained incidental to the irradiation program. In particular, the quantitative requirements of the growing rat and the adult rat for individual amino acids have been determined, and a method has been developed for the utilization of these requirements as a primary standard for the evaluation of protein. This standard can now be applied as one index of the effect of any type of processing on the nutritive value of the protein so treated. In this connection assay methods have been developed for the chromatographic determination of the amino acids in crude food materials, even those high in carbohydrates. This is particularly important in the case of methionine and cystine, which are very frequently the limiting food amino acids and which are normally difficult to determine in the presence of carbohydrate.

The major area of fundamental studies, however, has been that of vitamin K nutrition. Here methods have been developed for the production of vitamin K deficiency in the rat, which heretofore had not been believed to be dependent on a dietary source of vitamin K. The role of coprophagy has been determined, and methods for the purification of dietary ingredients, particularly protein, have been found which proves that on a truly K-free diet a deficiency is readily produced. A remarkable aspect of this study was the observation that only male rats were easily susceptible to dietary vitamin K deficiency. Female rats are relatively resistant to the deficiency and this has led to the discovery that estrogens possess "vitamin K like" activity in alleviating hypoprothrombinemia. The quantitative requirements of the weanling male rats

for vitamin K were shown to be the same on irradiated beef diets and on synthetic vitamin K-free diets, indicating the absence of any hemorrhagic compounds or antimetabolites of vitamin K in irradiated beef. Present studies are concerned with the function of vitamin K in intermediary metabolism, particularly with regard to its role in oxidative phosphorylation and the possible connection of oxidative phosphorylation and prothrombin biosynthesis.

MECHANISM OF THE HEMORRHAGIC PHENOMENON PRODUCED IN  
MALE RATS BY FEEDING OF IRRADIATED BEEF

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Medical College of Virginia

The initial aim of this project was a further investigation of reasons for the hemorrhage observed by Johnson in animals receiving certain diets containing irradiated beef with particular emphasis upon the apparent limitation of such to the male rat.

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and constituents of the diet and that irradiation of the food alone is not hemorrhage-producing.

2. In investigations of the role played by sex hormones in the hemorrhagic diathesis, it was demonstrated that castration greatly increased the susceptibility of the female rat but decreased that of the male animal. This has been confirmed by others.

3. Administration of estrogens has a protective effect, particularly in the male rat. Testosterone administration increases the susceptibility of the adult female animal to hypoprothrombinemia during the feeding of irradiated diets.

4. Not only the sex but the age of the animal is important in the particular response to the diets studied. The female becomes more resistant with maturity.

5. The sex and age differences noted with vitamin K-deficient beef diets was also found in situations in which K-deficiency was induced by means of an anticoagulant of the K-antagonist type. It was further shown that protection against the hypoprothrombinemia induced by the anticoagulant may be afforded by the prior or concomitant administration of estrogenic substances.

6. Important interrelationships exist between vitamins E and K. In repeated experiments, high levels of vitamin E administration (e.g. 400 mgm alpha tocopherol weekly) increased the likelihood of

MECHANISM OF THE HEMORRHAGIC PHENOMENON PRODUCED IN  
MALE RATS BY FEEDING OF IRRADIATED BEEF

Susan J. Mellette  
Department of Medicine  
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6. Important interrelationships exist between vitamins E and K. In repeated experiments, high levels of vitamin E administration (e.g. 400 mgm alpha tocopherol weekly) increased the likelihood of

hemorrhage in animals receiving K-deficient diets. At intermediate levels there is evidence that E may substitute for K under some conditions.

7. A depression of prothrombin occurs in the mouse during the feeding of irradiated beef diets similar to those used for the rat. Strain differences in prothrombin and in Factor V are apparent between C3H and Swiss Albino mice fed either laboratory chow or beef diets.

8. Not only do estrogenic hormones decrease the need for vitamin K, but vitamin K preparations have definite estrogenic effects in the rodent. Small doses of either  $K_1$  or menadione produce considerable increases in uterine weight in the castrate rat or mouse and induce vaginal cornification in such castrate animals. Conversely, high doses or prolonged administration results in decreased uterine weights.  $K_1$  has also been shown to decrease the effectiveness of estradiol when both are administered concurrently.

PATHOLOGY AND HISTOPATHOLOGY OF RATS  
FED IRRADIATED AND CONTROL FOODS

J. F. Bone, DVM, MS  
Department of Veterinary Medicine  
Oregon State University

A total of 860 rats are involved in this phase of the Irradiated Foods Project. Of this number, 102 are second and third generation animals and represent special examinations. The remaining 758 rats were divided into five experimental groups of 150 or more animals each, and were fed either pork, peaches, carrots, jam or flour, in addition to a complete basal ration. Since microscopic examination of the flour group is only one-third complete, the following report represents only the pathology of the groups fed pork, peaches, carrots and jam, covering a total of 608 animals.

The outstanding lesion which occurs in virtually all rats examined is lung damage due to chronic virus pneumonia. The reproductive tract, kidneys, middle ear, lymph nodes, adrenals and pituitary bodies, and the urinary bladder, represent the remaining organs which most frequently show lesions.

Reproductive tract pathology includes ovarian abscesses, ovarian and testicular tumors, metritis, seminiferous tubule degeneration, and uterine polyps. Kidney damage is principally due to senile nephrosis, chronic interstitial and glomerular nephritis, and polycystic degeneration. Lymphatic damage includes vacuolation, leukocyte infiltration, cyst formation and hyperplasia. Middle ear infection is non-specific. The

adrenals, except for a few tumors, show hemorrhagic cysts in the cortex. The pituitary bodies show hemorrhage into the anterior pituitary and chromophobe adenomas. The urinary bladders show a low but constant incidence of Trichosomoides crassicauda parasitism. The remaining general pathology is of a sporadic nature.

Tumor pathology is extensive. Malignancies average 6.9% for 304 males and 5.3% for 304 females in the 608 animals. No specific malignant tumor occurred frequently enough for comment. Non-malignant tumors averaged 8.2% for 304 males and 18.75% for 304 females, the bulk of this difference being reflected in mammary adenofibromas and uterine polyps. The high overall incidence (15.1% in males and 24.05% in females) can probably be attributed to the fact that this has been a long-term survival study and that many of these tumors can be associated with old age. The highly inbred strain of rats used may also exert some effect on tumor number.

Considering all neoplasms, carrot, peach and jam groups showed a higher percentage of neoplasms (carrot 55%, jam 60% and peach 80%) than the animals fed pork. In malignancies, the peach diet showed by far the largest number of neoplasms (15) when compared with pork (7), carrot (8), and jam (7). In benign neoplasms, carrot (23), peach (21) and jam (25) diets, all were notably higher than pork (13). A sex incidence was observed in both benign and malignant tumors. Males showed a total of 21 malignancies compared to 16 in the females. Females showed a total of 57 benign tumors compared to 25 in the males. Animals on the 6 megarad

levels showed a significantly higher incidence of malignant tumors than did the controls or those on the 3 megarad levels. The same pattern was noted in benign tumors, but the numerical difference was not so pronounced. Animals on the 3 megarad levels showed the lowest tumor incidence of all groups.

PATHOLOGIC CHANGES IN RATS FED CHICKEN AND GREEN BEANS

R. H. Rigdon  
Professor of Pathology  
The University of Texas Medical Branch

Observations have been made on the pathologic changes in 92 rats, 50 males and 42 females, fed diets containing irradiated and non-irradiated chicken and green beans by Dr. L. R. Richardson at Texas Agricultural Experiment Station, College Station, Texas. The most significant pathologic changes observed are shown in the following table:

<u>SUMMARY OF LESIONS IN RATS FED CHICKEN AND GREEN BEANS</u>										
DIET	1	2	3	4	5	7	9	M	F	TOTAL
NO. OF RATS	42	3	16	1	5	11	16	50	42	92
Males	24	3	8	1	2	5	7	50		50
Females	18	0	6	0	3	6	9		42	42
Lung-Infection	39	3	14	1	4	10	15	48	38	86
Stomach-Ulcers*	6	0	1	0	1	0	0	5	3	8
Pancreas-Vacuoles	15	1	2	1	0	5	10	21	13	34
Kidney-Calcium**	6	0	4	1	0	3	9	6	17	23
Bladder-Calcium	1	0	0	0	1	1	0	1	2	3
Bladder-Concretions	2	0	1	0	1	1	1	6	0	6
Bladder-Precipitate	12	0	2	0	0	0	1	15	0	15
Heart-Endocarditis	4	0	0	1	0	0	1	2	4	6
Uterus-Endometritis	10	0	4	0	1	1	2	0	18	18
Pituitary-Adenoma	2	0	0	0	0	2	0	0	4	4
Bladder-Papilloma	1	0	1	0	1	1	1	1	4	5
Breast Adenocarcinoma	1	0	0	1	0	1	0	1	2	3
Lymphosarcoma	2	0	0	0	0	1	0	3	0	3

\* One of these ulcers occurred in the duodenum

\*\* One rat had a stone in the ureter

For the specific diets see report by Dr. L. R. Richardson

No rats fed Diets 6 and 8 in this group

In addition to the incidental finding of pulmonary infection in these rats, many had calcium-like material in the renal pelvis and in

the lumen of the renal tubules. Calcium was present in the urinary bladder of 7 rats. The calcium sometimes occurred as gross calculi and at other times only as a microscopic precipitate. An eosinophilic granular precipitate and concretions occurred in the bladder of male rats (Published paper: "Concretions in the bladder of male rats," The Journal of Urology, 84:307-310, 1960). Neither the calcium or the eosinophilic precipitate appeared to be related to the intake of irradiated food. There were no changes in the bone marrow to suggest a pathologic process.

From this study there are no changes observed that we can associate with irradiation. The most frequent pathologic change observed was a pulmonary infection. The characteristic of this infection was similar in the different rats; however, there was a variation in the degree of pulmonary involvement. Both benign and malignant tumors were present, but no metastases were observed. Vacuoles in the epithelial cells of the pancreatic acini were observed frequently in this study.

## RADIOISOTOPES IN RADIATION PROCESSED FOODS

G. R. Meneely and Raymond L. Weiland  
Vanderbilt University

The first phase of Vanderbilt University investigation of radioactivity in irradiated food was a review of the sample measuring performance of the steel shielded counting chamber, the function of the associated gamma counter and multichannel pulse height analyzer, preparation of the gamma emitting isotope standards, operational procedures, and the programming of our IBM Computer. Reproducible geometrical counting arrangements were established for counting food samples in No. 10 and No. 2 cans. Background radioactivity measurements have been made using cans filled with triple distilled water, unirradiated pork, ham, beef and chicken. The gamma-ray spectra obtained from the unirradiated foods did not differ from those of normal humans, i.e., the only significant gamma-ray photopeaks from 0 to 6 Mev are cesium-137 and potassium-40.

No induced radioactivity could be detected in raw pork irradiated by cobalt-60 or by 8 or 11.2 Mev electrons. Induced radioactivity was found in raw pork irradiated by 12, 13 and 16 Mev electrons. In each instance prominent photopeaks were present. The quantity of induced radioactivity increased with increasing energy of the electron beam. The same isotopes were present in each batch. A preliminary identification of these indicates the presence of Na-24, Sc-46, Ta-182, Ir-190,

Ag-106, Ba-135m, Rh-101m and Pt-193m. The threshold for activity detectable by gamma-ray scintillation spectrometry induced by electron irradiation thus appears to lie between 11.2 and 12 Mev. Beta particle analyses of these samples also show some increases in detectable levels of activity attributable to P-32 and H-3 without significant evidence of increase in P-33, S-35 and C-14 activity.

The largest quantity of gamma rays detectable after the above described irradiation is less than ten times that detectable from unirradiated food.

SUMMARY REPORTS OF TECHNICAL SESSIONS

1. Biochemistry of Sensory Changes	A. L. Tappel
2. Container Technology	G. Pratt
3. Dosimetry and Dose Distribution	R. A. Glass
4. Microbiology	L. L. Kempe
5. Product Technology	R. F. Cain
6. Wholesomeness and Nutritional Adequacy	L. R. Richardson
7. Heart Lesions in Mice	S. W. Thompson
8. Hemorrhagic Syndrome	B. C. Johnson
9. Histopathologic Findings	R. H. Rigdon
10. Carcinogenicity Studies	B. E. Kline
11. Human Feeding Studies	M. E. McDowell
12. Other Radiation-Induced Changes	I. J. Tinsley

## BIOCHEMISTRY OF SENSORY CHANGES

A. L. Tappel  
University of California

Discussions involving biochemistry of sensory changes focus on sensory changes in meat products. There are two main lines of study which have been pursued during the past two years. These include chemical identification of irradiation-produced compounds formed in meat and the irradiation chemistry of the precursor compounds to try to account for these on a chemical basis. The compounds formed from the precursor include organic carbonyls, usually of small molecular weight (2 to 8 carbon atoms), a variety of sulfur compounds including compounds like hydrogen sulfide, methylmercaptan, dimethylsulfide, etc., and also some compounds we are not able to account for on the basis of known chemical reactions. These include aliphatic and aromatic hydrocarbons. Among the first group of compounds, the carbonyls and sulfur compounds, irradiation chemistry looks rather straightforward as such things go. The carbonyls arise mainly from the lipid in the meat and sometimes from the amino acid. The sulfur compounds derive mainly from amino acids, small peptides and proteins. These incidentally are not unusual compounds to be found in food products, and there is no question concerning their safety. The main question of course concerns their odor and flavor. Since there is considerable difficulty in identifying odor and flavor constituents by nose only, most biochemists would choose to use modern methods of gas

chromatography and other methods well established in the chemical sense. Another problem concerns the textural changes caused by irradiation of meat. There are several possible chemical explanations such as changes in the water binding capacity of the proteins and depolymerization of proteins, both of which seem like logical reactions. In conclusion, you might say that all of these studies on the biochemistry of meat are in fact pioneering studies in the sense that they have never been done before, and so we are learning a great deal about the biochemistry of meat. There do not seem to be unusual problems other than the odor and flavor changes encountered in the irradiation of meat.

## CONTAINER TECHNOLOGY

George Pratt  
American Can Company

The problems which were discussed in the container section reflect the amount of progress made since the beginning of the program. In addition to having a choice of rigid containers, we have a selection of metals, enamels, and can-lining compounds which we think will be good for the whole gamut of irradiation food products. Those problems which took up a good deal of the meeting time pertained to eventual approval by The Surgeon General and the Food and Drug Administration. This is an advanced problem when you realize that we have not yet received final clearance from the Food and Drug Administration for the tin can presently in use for thermal processing. However, our extractive studies with irradiated enamel lined cans demonstrate that the amount of extractives is low and of the same order of magnitude as in thermal processed cans.

In addition to metal cans we would also like to have flexible containers. At present, no plastic film is perfect from the standpoint of physical properties and resistance to radiation. A ray of hope is generated by the development of antioxidants (anti-rads) which show promise of minimizing the development of odor and extractives in polyethylene during irradiation. Currently, these antioxidants are being explored in greater detail. The ultimate flexible container will be a multi-ply combination, consisting of a layer for sealing, a layer providing moisture and oxygen protection, and finally a layer or combination of layers for strength.

The extractive studies should be pursued with vigor. In the past, toxicity studies could not be conducted on packages because of complicating factors associated with irradiated food. Now we know enough about these foods to produce nutritionally adequate diets and therefore be able to determine the effects of the packages without complicating factors.

There is also need for realistic physical tests to evaluate flexible containers. We must know how much damage a package can sustain without losing its protective qualities, and then relate this information to actual shipping conditions.

## DOSIMETRY AND DOSE DISTRIBUTION

Richard Glass  
Stanford Research Institute

Recently only a very modest amount of work has been conducted in the dosimetry and dose distribution fields, largely because these situations are pretty well under control, and not much additional work is needed.

The standard dosimeters remain ferrous-sulfate solution (primary) and ceric-sulfate solution as well as cobalt glass (secondary). The important remaining problem is to sift and distill dosimetry developments and procedures. The ultimate aim of this screening is a compact handbook incorporating in step-by-step form, the selected dosimeter application techniques. Among other problems are the simulating of food or other sample geometry by means of phantoms, and the effect of plastic cells on dosimeter response.

Several new dosimeters were discussed, including those based on measurements of resistance, capacitance, viscosity, electron-spin resonance (EPR), and induced fluorescence (Ag phosphate glass). At the present time, none of these have apparent great advantages over the systems adopted by the Quartermaster Corps.

Dr. Paul Feng briefly reported his dose-distribution studies. Relatively small dose increases have been observed at interfaces such as at food-can walls. Backscattering and other effects can explain the phenomena, however. The dose increases only exist in the region within several millimeters of the can surface and do not constitute a large perturbation of overall dose depth patterns.

## MICROBIOLOGY

Lloyd L. Kempe  
University of Michigan

Summarizing the discussion of the bacteriology section is something less than easy. I think most of you recognize that we couldn't stop in the allotted length of time and still include everything we wanted to talk about. There were at least two sides to every question, and each side had its own supporters. However, I have summarized about half a dozen points that I think are important.

Use of the 12D concept results in calculated radiation sterilization levels which are approximately equal to those which produce sterile canned meats by means of the end-point method (which utilizes inoculated packs). In other words, both methods produce essentially the same results. However, in planning an inoculated pack, it must be presumed that if one viable anaerobic spore is present in a can of unpreserved meat, this spore may possibly grow and develop into a culture. This statement stems from the discussion surrounding the probability of using, for example, 10,000 spores in one can or one spore in 10,000 cans. There was disagreement about the similarity of results based on these two concepts. Discussions revealed that the usual putrefactive anaerobe concentrations in meat are less than 0.1 per gram. The ratio of P.A. spores to botulinum spores is thought to be about 100 to 1.

Classical survival curves for bacterial spores are drawn in a straight line. We all recognize that radiation survival curves, that is,

percent survivors plotted against radiation dosage, do not really agree with the classical curve. There is a hump at the beginning, and a tail has been observed at the end. The tailing-off effect does not alter our current thinking regarding radiation-sterilization levels since these have been largely based upon end-point analyses. The resulting sterilization values are almost identical with those observed at the end of the tails.

In view of the increasing recognition of Clostridium parfrigens as a food poisoning organism both in this country and abroad, some studies regarding radiation sensitivity of this organism are in order.

Contractors were again reminded to carefully specify details such as dose rate, temperature for irradiation, and the like, in their requests for irradiation, in order that the results may be meaningful.

It was agreed that simultaneous pursuit of applied and basic bacteriology research should be carried out on anaerobic bacteria and preferably on Cl. botulinum organisms since these are of most interest to the Quartermaster Corps Radiation Preservation of Foods Program.

Lastly, such terms as "radiation sterilization" and "radiation pasteurization" can be used with meaning but must be clearly defined for each situation reported by the user.

## PRODUCT TECHNOLOGY

Robert F. Cain  
Oregon State University

Interesting discussions on the present "state of the art" revealed several points of agreement, disagreement and focal points for continued research.

1. Texture: There was diverse opinion concerning the effect of radiation on the texture of poultry. One group reported that radiation caused a toughening of the meat similar to that encountered when fried chicken is frozen, while another group reported a tenderization effect under similar radiation treatment. In beef, texture remains a problem. The grade of the carcass is important in that "canner" grades show less tenderizing effects following radiation than choice grade, although unanimity of opinion on this point was not reached.

The method of enzyme inactivation is very important in its effect upon texture. Several workers reported beneficial effects on texture following high temperature/short time enzyme inactivation procedures.

Trends in results indicate that high fat content and long time inactivation procedures promote a less desirable texture in pork. English workers reported more desirable texture occurred when less tender cuts of pork and beef were enzyme-inactivated prior to irradiation. It was interesting to note the unanimity of opinion with respect to the use of high temperature/short time enzyme-inactivation procedures for beef, pork and poultry.

2. Color: The group did not feel that the color which developed upon irradiation of enzyme-inactivated beef, pork or poultry was of

major significance with respect to the acceptability of the product. An improvement in the color of enzyme-inactivated poultry can be obtained by packaging in air and increasing the blanching temperature to 185° F. internally, as opposed to bringing the product to an internal temperature of 155° F. for 45 minutes.

3. Flavor: General consensus of opinion was that storage at room temperature resulted in a diminution of the irradiated flavor. Deep-fat-fried pork, beef, and chicken have less radiation flavor and were preferred over samples cooked by other methods when the cooking was accomplished after radiation. A notable decrease in the radiation flavor results when beef is radiated at liquid nitrogen temperatures.

The grade of carcass and type of muscle used does not influence the intensity of irradiated flavor. Rancidity appears not to be a problem in irradiated pre-cooked beef, probably due to a suggested antioxidant.

4. Additives: With respect to radiation odors, charcoal packets appear to be effective in reducing the intensity of the radiation odor in pork, chicken, and crab. Ascorbates and nitrites appear to reduce the intensity of the radiation odor by combination, while barbecue sauces, liquid smoke, salt, pepper and other condiments improve acceptability scores of meat probably by exercising a masking effect. Holding fresh beef under nitrogen overnight prior to enzyme-inactivation and radiation improves acceptability scores and reduces radiation flavor probably by oxygen consumption.

Antioxidants appear not to affect radiation flavors but assist in reducing the rancid flavors that develop during storage.

A number of radiation sterilized products are now within the

acceptability range but require field testing. Specifications can be written for a number of products, but evaluations by highly critical panels leave doubt as to where we are in the product development field. We seem to be stopped someplace along the line and what we need now in order to move on, is to get some field testing on these products to establish actual acceptability range and scores by the final military consumer.

WHOLESOMENESS AND NUTRITIONAL ADEQUACY STUDIES

L. R. Richardson  
Texas A&M College

The results of long-term feeding studies conducted on 20 foods over a two year period were reported. These foods were fed to dogs, rats, mice, and monkeys. Irradiation doses ranged from pasteurizing and sprout inhibiting levels to sterilizing doses (2.78 and 5.58 Mrad).

Hemoglobin values, red-cell counts, white-cell counts, differential counts, organ weights, and fertility studies were used as means for measuring the value of the food. There was no toxicity observed which could be attributed to irradiated foods. There was no difference in nutritive value between the control and irradiated foods, which could be attributed to the radiation process when these foods were supplemented with vitamins that are known to be destroyed or reduced by irradiation.

## HEART LESIONS IN MICE

Samuel W. Thompson, Lt. Col., VC  
USA Medical Research & Nutrition Laboratory  
Fitzsimons General Hospital

In 1957, Dr. Harry Monsen initiated a study to determine the carcinogenic effect, if any, of a composite diet composed of five irradiated foods and using two strains of mice, Cb and Strong A. In 1958, in the course of his investigations, he observed that a large number of his animals were becoming ill. Upon their sacrifice, it was noted that a sizable number of these animals had developed a rather peculiar lesion, a dilatation of the left auricular appendage, some of which ruptured. This condition was new not only to Dr. Monsen but also to other investigators. Since 1958 he has observed no new cases occurring in the animals on his study until about 600 days on test. The original lesions occurred after an average of 85 days on diet. At 600 days on diet he has noted that, of the total number of experimental animals, nearly 50% of the Cb strain on the irradiated diet had such lesions, as compared to a far less significant figure among the controls.

While attempting to investigate this finding, he fed both irradiated and non-irradiated milk, and was able to reproduce the lesion in the Cb strain with both diets.

It was then decided to repeat the experiment with a view to studying this particular condition, since Dr. Monsen's original project was a carcinogenic study, (a lifetime effect), so that the test animals could not very well be sacrificed. The repeat study was set up with

some 5,000 animals in the feeding program. All phases of the study are on test, and all phases of Dr. Monsen's original experiments are, we hope being simulated. The second experiment, however, was designed so that the animals could be sacrificed at periodic intervals throughout the life span. This will enable us to evaluate how and when the lesions develop.

It was contemplated that this condition might be due to dietary stress associated with a genetic aspect. Therefore, extensive records are being maintained on the repeat experiment as to the breeding program (sires and dams) and the animals on test are individually identified.

Some 1800 animals on the repeat experiment have been sacrificed, and the tissues of 400 examined. Lesions have been observed in the hearts of about six, and one lesion was detected macroscopically. These were mural thrombi with associated myocardial degeneration and calcification. Three were observed in the left auricle, two in the right atrium and one in the coronary artery. While three of these are suggestive of Dr. Monsen's lesions, we are far from the date at which he observed the great proportion of affected animals. According to present schedules, the repeat experiment should be completed in September 1962.

## THE HEMORRHAGIC SYNDROME

B. Connor Johnson  
University of Illinois

To those of us involved in the study of vitamin K deficiency in animals previously not thought to develop it by dietary means, one of the most interesting parts of The Surgeon General's program has been the follow-up of this problem.

About four years ago, we first observed hemorrhage in rats fed irradiated beef. Later on, various investigators found that rats fed the control (nonirradiated) beef also developed hemorrhages, but less frequently than those on the irradiated ration. Unirradiated pork also produced the disease. In a completely different diet, various proteins were found to effect it. The disease was not brought about by some types of vitamin-free casein, but it could be induced by others. Most recently, the vitamin K deficiency resulted from the feeding of amino acid diets; a hemorrhagic compound occurred in the protein supplement used. This shows very conclusively that there is nothing hemorrhagic, nothing antagonistic to vitamin K in irradiated beef.

Coprophagic effects also have to be ruled out, although we know very well that coprophagy delays the disease symptoms. If coprophagy is prevented, the disease appears much more rapidly; in fact the animals will have prolonged prothrombin times in three or four days. Reports of various strain differences among rats complicated the early work. We now know we can produce the disease in all strains of rats and a

susceptible mouse strain has been found. Recently, the disease was produced by dietary means in young pigs, and work on monkeys has started.

We have found, by means of  $C^{14}$ , that vitamin K absorption is not involved.  $K_3$  shows that an irradiated diet has no effect on absorption. Today however, it was reported that, in the case of  $K_1$  ( $C^{14}$ ), 10-12% is normally absorbed from the irradiated dietary. The stability of vitamin K itself to radiation varies according to source and site. In fruits and vegetables, vitamin K is stable. A pure solution of  $K_3$  is relatively stable when dissolved in oil. On the other hand, irradiated beef loses one-half to two-thirds of its vitamin K on irradiation, as measured by chick assay. Many investigators working in the vitamin K field for years have known that  $K_3$  is not stable as mixed in ordinary diets.

Other dietary factors involved in the vitamin K problem have proved to be very embarrassing. Vitamin A particularly has shown a straight-line relationship between level of A and vitamin K requirement, even down to levels of vitamin A which are in the normal range of requirement and feeding. This was certainly a most unexpected finding; it has been found recently that vitamin A acid is even more antagonistic to vitamin K than the usual alcohol or ester.

It has also been found that vitamin E is antagonistic to vitamin K. Increased levels of vitamin E correspondingly enlarge the K requirement.

The protective effect of methionine is another most interesting phenomenon. We have known from past work that methionine is the amino acid in protein most sensitive to radiation. Perhaps this is merely a matter of methionine being required for protein synthesis, prothrombin

being a protein. At any rate, the effect of methionine on the K requirement is very noticeable.

From the very beginning, it has been shown that the male rat is susceptible to the disease to a considerably greater extent than is the female rat. On the other hand, chicks show no difference in susceptibility between the sexes. Castration makes the male rat more resistant to the deficiency, and the female less resistant. The same effects are noted when anti-coagulants are administered. The recent findings that estrogen administration will protect a male rat while testosterone will increase the hemorrhagic incidence in the male and bring it about in the female, are most surprising. An even more astonishing event occurs when you treat a male animal who is dying of vitamin K deficiency. Prothrombin activity is restored in about four hours after the administration of vitamin K. The same restoration occurs if estrone is given. The estrogen appears to cure the animal just as does the vitamin. It is just as surprising to note that vitamin K exhibits an estrogenic response similar to that given by an estrogen.

Two other factors were discussed. Factor V or accelerator globulin is one of a great number of components involved in the blood clotting cycle. Dr. Mellette early reported that while prolonged prothrombin times were cured by administration of vitamin K, Factor V depression, brought about by eating irradiated foods, did not respond to this treatment. This anomaly has not yet been completely resolved. There is still no consistent correlation between Factor V and prothrombin. Since, however, Factor V levels are increased by methionine, the effect of radiation on methionine must be elucidated.

Lastly, we have assimilated a great deal of recent work on the molecular level function of vitamin K involving amino acid incorporation in the protein depressed by vitamin K deficiency. It appears that protein synthesis may be involved in the reaction as well as oxidative phosphorylation.

This is the status of the work at the moment.

## OTHER RADIATION-INDUCED CHANGES

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Although some differences due to the irradiation process have been observed in the rate and extent of digestion of different macronutrients, the treatment usually does not have a very significant effect. In some cases, radiation has increased the rate of digestibility while elsewhere digestibility decreased. In most cases the order of these effects is not very large, so that the effect on the absorption of a particular food component is not considered to be very significant.

However, it has been observed that the rate of absorption of irradiated lipids, particularly lard, is quite significantly retarded in dogs, and as well in rats when measured after a prolonged feeding period.

According to the results of tissue enzyme studies, there are probably two areas under observation. In some studies on blood enzymes, changes in enzyme activities have been observed which can be directly related to vitamin deficiencies, particularly thiamine and pyridoxine deficiencies. These two vitamins are affected by irradiation processing and so in unsupplemented rations tissue enzyme effects corresponding to the level of the vitamins present in the rations are obtained. These effects of course can be alleviated by supplements of these vitamins. Changes in enzyme activities in other tissues have been observed, non-specific changes which are not, as far as is presently known, the result of a vitamin deficiency. These changes have been observed in animals which were raised on supplemented rations and their exact explanation and significance in this program is hard to interpret at the present time.

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