TOXICOLOGICAL SAFETY OF IRRADIATED FOODS[1]

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INTRODUCTION

The treatment of foods with ionizing radiation has been intensely investigated for the past two decades. Although the concept of prolonging the useful storage life by exposure to X irradiation was patented as early as 1930 (1), feasibility studies on this novel method of processing were not undertaken until shortly after World War II. Early studies in the United States and Great Britain demonstrated a number of possible industrial applications on a theoretical and laboratory basis (2). Because of the potential logistic advantages of preserving food for long periods of time without the benefit of refrigeration, the U. S. Army Quartermaster Corps embarked on an extensive radiation sterilization of foods program in 1953. Concurrently, interests in certain advantages of food irradiation became well established in a number of countries.

Terms of reference.—The major potential applications of food irradiation which have become apparent are summarized as follows:

1. Pasteurization—the prolongation of storage life of foods in their fresh state by inactivation of the vegetative forms of spoilage bacteria, fungi, etc.
2. Sterilization—the stabilization of foods for long-term storage without refrigeration by inactivation of all vegetative forms of bacteria, fungi, etc., and by complete inactivation of all resting spores of public health significance.
3. Disinfection—the inactivation of certain pathogenic microorganisms (e.g., *Salmonella*) in foods.
4. Disinfestation—the sexual sterilization of various insects or parasites in bulk or packaged forms of food.
5. Sprout inhibition—the suppression of sprouting in bulk stored potatoes and root crops.

More detailed potential applications of food irradiation are listed in Table I (3). Several new and more exacting terms for food irradiation applications have recently been proposed by an international expert committee (4): Radappertization (radiation treatment to achieve "commercial sterility"); Radicidation (radiation treatment for destruction of pathogenic organisms); and Radurization (radiation treatment for prolonging storage life).

[1] The survey of the literature pertaining to this review was concluded in July 1966.

TABLE I

SOME POSSIBLE APPLICATIONS OF IONIZING RADIATION TO THE TREATMENT OF FOOD

Group	Food	Main objective	Means of attaining objective	Dosage (Mrad)
(*a*)	Meat, poultry, fish, and many other highly perishable foods	Safe long-term preservation without refrigerated storage	Destruction of spoilage organisms and any pathogens present, particularly *Clostridium botulinum*	4–6[a]
(*b*)	Meat, poultry, fish, and many other highly perishable foods	Extension of refrigerated storage below 3° C	Reduction of population of microorganisms capable of growth at these temperatures	0.05–1.0
(*c*)	Frozen meat, poultry, egg, and other foods liable to contamination with pathogens[b]	Prevention of food-poisoning	Destruction of salmonellae	0.3–1.3[c]
(*d*)	Meat and other foods carrying pathogenic parasites	Prevention of parasitic disease transmitted through food	Destruction of parasites such as *Trichinella spiralis* and *Taenia saginata*	0.01–0.03
(*e*)	Cereals, flour, fresh and dried fruit, and other products liable to investation	Prevention of loss of stored food and spread of pests	Killing or sexual sterilization of insects	0.01–0.05
(*f*)	Fruit and certain vegetables	Improvement of keeping properties	Reduction of population of molds and yeasts and/or in some instances delay of maturation	0.1–0.5
(*g*)	Tubers (for example, potatoes), bulbs (for example, onions), and other underground organs of plants	Extension of storage life	Inhibition of sprouting	0.005–0.015
(*h*)	Spices and other special food ingredients	To minimize contamination of food to which the ingredients are added	Reduction of population of microbes in the special ingredient	1–3

[a] There is evidence that a lower dose might suffice for certain cured products.
[b] Including animal feeds.
[c] A higher dose may be needed if pathogens with greater resistance to radiation are present.

As technological advances were made in these areas, it was recognized that extremely stringent tests relating to the public health safety of this novel principle of food preservation were necessary. The purpose of this paper is to discuss the paramount findings in relation to the "wholesomeness" or "safety for consumption" of irradiated foods. In a broad sense, these terms refer to the following major areas of investigation:

1. Toxicological safety—evaluation of the formation or introduction of harmful substances as a result of exposure of food to ionizing radiations.
2. Nutritional safety—evaluation of the possible degradation of nutrients which are of essential importance in the diet.
3. Microbiological safety—evaluation of the presence of harmful microorganisms, toxins of microbiological origin, or both in irradiated foods.

Principles involved in preservation of food by ionizing radiation.—The preservation of food by any processing method is based primarily on the destruction or inhibition of the growth of microorganisms and by inactivation of autolytic enzymes. Ionizing radiations are capable of destroying all known types of microorganisms in foods, provided that the food material in which the organisms are located is within the physical penetration limits of the particular type of radiation used (5). The various species of microorganisms vary greatly in their natural radioresistance characteristics. Bacterial spores and viruses are normally far more radioresistant than the vegetative forms or organisms. Factors such as temperature, moisture content, atmosphere, and chemical additives may also affect the resistance of organisms. The radiation dose required to achieve a desired microbiological objective also depends on the number of organisms likely to be present before irradiation and the number of viable organisms that can be tolerated in the final irradiated product.

Ionizing radiations possess the capability of penetrating matter and causing ionization to occur. Energy is absorbed by the food in this process. This energy transfer may destroy living microorganisms and at least part of the natural autolytic enzymes in the food in a direct manner (first order exponential function as with thermal energy) by striking sensitive portions of the cells and causing their death, or in an indirect manner, by the action of extremely short-lived and reactive free radicals. In addition, numerous other chemical changes may result from the ionization processes. If certain of these chemical changes are excessive, undesirable flavor, odor, and texture changes may occur in the food.

Types of ionizing radiations which possess suitable characteristics for food irradiation include gamma rays, X rays, and high speed electrons. Gamma rays and X rays are electromagnetic radiations which possess the same physical characteristics and cause similar biological and biochemical effects. X rays are machine generated, resulting from the impingement of fast electrons on a target, while gamma rays are emitted during the decay process of isotopic sources such as cobalt-60 and cesium-137. Electron radiation is generated by machine sources (accelerators).

Requirement for government supervision and control.—In order to provide adequate health safeguards for the individual consumer, it is essential that legislation regarding the production, importation, exportation, and distribution of irradiated food be established and rigidly enforced by governments. Government authorities must carefully evaluate all data related to the health aspects of each irradiated food before approval for its production and public consumption is officially granted. Petitions for the unlimited public consumption of an irradiated food must be supported by appropriate data which clearly demonstrate the absence of toxicity, carcinogenicity, and microbiological hazards and attest to its nutritional adequacy. Food irradiation facilities must be properly licensed and regulated and governmental inspection of the production, handling, and labeling declarations of irradiated foods will be necessary.

To promote the future international trade of wholesome irradiated foods, it is necessary that the various countries make every attempt to coordinate and standardize legal requirements concerning the production and use of irradiated foods. Periodic international meetings of experts on food legislation from various countries, such as the FAO/WHO/IAEA meeting in April 1964 (3), are extremely desirable for coordinated efforts toward the attainment of this goal.

CONSIDERATIONS ON EFFECT OF RADIATION ON CHEMICAL COMPOSITION AND NUTRITIVE VALUE

Comparable to all food preservation methods, the adaptation of ionizing radiation for lethal action on spoilage microorganisms can be expected to produce certain changes in the molecular structure of organic compounds present in the food, with the concomitant reduction in nutritive value. There may be some qualitative and quantitative differences relevant to sterilization of foods by thermal or radiation treatment, but nutrient losses are in general quite similar. Whatever method is used for preservation, there is an additional loss in nutrients when foods are heated preparatory to consumption. For shelf life extension or radiation pasteurization of foods, usually at levels below one million rads, the chemical changes and alterations in micronutrients and macronutrients are not readily affected.

The chemical changes induced by radiation preservation are revealed by certain color, odor, and flavor changes in foods, especially at radiation sterilization dose levels from 2 to 5 million rads (Mrads). Investigations on these subtle chemical changes have been directed toward elucidation of mechanisms of action of radiation on the major food components: lipids, proteins, and carbohydrates.

Proteins.—In spite of the fact that few of the chemical bonds in materials irradiated have been broken, these alterations in case of proteins include denaturation, degradation, and polymerization. Proteins show the influence of aggregation and increase in viscosity of irradiated protein solutions. Irradiation of milk protein at high levels (6 to 10 Mrads) results in a marked alteration in serologic activity or reduction in antigenicity (6). Irradiation of free amino acids or peptides results in deamination and decarboxylation. The amino acids in proteins, as they occur in peptide linkages, are more resistant than the amino acids in free solution (7). Some proteins, such as beef protein, may yield ammonia and at least six amines, predominant among which are methylamine and ethylamine (8). The sulfur-containing amino acids yield methyl mercaptan, hydrogen sulfide, methyl disulfide, and isobutyl mercaptan (9). These products are odoriferous and bad tasting, which characterizes the adverse properties of some irradiated foods. Certain carbonyl compounds have been identified, such as 3-methyl-thio-propionaldehyde, which has been shown to be a component of at least 12 substances identified as volatile constituents produced in concurrent radiation distillation at 5 Mrads in processing raw beef (10). A Maillard type reaction may occur on irradiation of proteins in foods.

Some amino acids have shown losses of glutamic acid and serine after radiation processing. Quite significant is the fact that environmental factors, such as presence of salt and oxygen, temperature and pH, influence radiation damage in proteins. Hence, extrapolations cannot be made from model systems to whole food.

Up to 10 Mrads of radiation dose there is little effect on the digestibility or biological value of food proteins. However, the loss of biological value of milk protein at high doses (6 Mrads and above) is due to sulfur amino acid destruction (11). The degradation products of proteins or amino acids are apparently nontoxic, as evaluated from animal feeding experiments. Since the profound changes in proteins are in the physical and biological characteristics, more basic information is required on effect of radiation on proteins *in situ*.

Most of the enzymes, especially the proteases in foods, are resistant to high doses of radiation, requiring up to 20 Mrads for complete inactivation. This property distinguishes radiation processing from thermal processing and, indeed, in current radiation technology certain foods receive enzyme inactivation by blanching prior to radiation sterilization.

Lipids.—The radiation effect on fats is similar to that of autoxidation with hydroperoxides among the initial products of irradiated fats. The reaction chains in the formation of peroxides during irradiation are shorter than in autoxidation since free radical concentrations are higher. The radiation dose determines the concentration of peroxides, with more peroxides formed at the lower dose rates. In further decomposition of peroxides, reactive carbonyl groups, such as ketones and aldehydes, are formed even under anaerobic conditions. Irradiated, unsaturated fatty acids show changes in the double bond configuration. Contrasted with irradiation effects on proteins, the lipids show insignificant alteration in physical properties such as melting point and dielectric constant even at high radiation doses (12).

Animal fats are more susceptible to radiation-induced chemical changes than vegetable fats, but these can be reduced by use of low temperature and exclusion of oxygen. Whereas hydroperoxides were considered earlier as the potential toxic compounds formed in irradiated lipids, this concept is now debatable (13). Whatever the nature of these oxidized lipids, which have physiological action in experimental animals (14–17), they are only formed when peroxide numbers of fat reach 100 or above, which is not attained at radiation sterilization doses of 2.8 to 5.6 Mrads (18). The oxidative effect of irradiation on lipids may involve transformation of essential fatty acids, inducing nutrient deficiency (19). The carbonyls which are formed are known at least to destroy some fat-soluble vitamins, especially vitamin E, and, as will be mentioned later, are responsible for vitamin K inhibition. When irradiated at 5 Mrads, the rate of digestion and absorption of fats was slightly reduced, but in general this has no nutritional significance (20).

Carbohydrates.—The effects of ionizing radiation on carbohydrates are not as well delineated as those on proteins and lipids. Hexoses are degraded by dehydrogenation, and complex polysaccharides exhibit a break in the glycosidic linkage. The effects induced by irradiation are continued during storage conditions, dependent on the presence of water and on temperature. The properties of carbohydrates are altered slightly, such as loss in gelation and some browning, the latter is accentuated in the presence of protein when interaction produces polymers (21).

The metabolizable energy or nutritive value of carbohydrates is not appreciably affected. Some Swedish workers have drawn attention to a "radiomimetic effect," and there is also under consideration a potential gene mutation and rearrangement of chromosomes attributed to irradiated carbohydrates (22). The radiomimetic effect disappears when irradiated substances are heated (23). It is possible to degrade cellulose to digestible sugars by radiation doses up to 100 Mrads. Free sugars can be formed from carbohydrates at radiation sterilization doses (3 to 6 Mrads).

Vitamins.—The radiosensitivity of the micronutrients, either the water- or fat-soluble vitamins, varies considerably and the destructive effect of radiation on vitamins in foods differs from that in pure solution. The nature and physical state of the medium and the radiation dose applied determine the degree of destruction. The destructive effect of radiation may be indirect in that the free radicals in the solvent or oxidized compounds, such as peroxides and carbonyls, in solid medium react with the vitamins and other biologically active materials. In experimental animal response to administered irradiated foods or diets, it must be ascertained therefore whether observed physiological response is truly a manifestation of a toxic reaction to an irradiated end product or a reflection of a nutrient deficiency induced by radiation processing.

Of the fat-soluble vitamins, E and K are quite radiosensitive, with vitamin E being the most labile. Vitamin A and carotene are radiosensitive, dependent upon the media or food in which these nutrients are suspended. Since it has been pointed out that lipids form peroxides when irradiated, in a food substrate these peroxidized compounds have an antagonistic effect upon the antioxidants. In this case, α-tocopherol, an antioxidant and an essential vitamin in reproductive function, is readily destroyed. As early as 1948, it was observed that in feeding a diet exposed to capacitron irradiation, female rats exhibited reproductive failure (24). To obviate this effect, in toxicity investigations on irradiated foods, diet supplementation of vitamin E has always been practiced.

Perhaps extensive toxicological investigations on the hemorrhagenic effect of feeding irradiated beef diets have had an indirect accomplishment in that this research program has done much toward elucidation of vitamin K metabolism. Estrogens apparently decreased the need for vitamin K since male rats, fed irradiated beef diets and not practicing coprophagy, were quite susceptible to K deficiency and hemorrhagic diathesis. Not only sex, but the age of the animal, is important in this disease syndrome since male rats are more susceptible than weanling male rats (25). Beef treated at 6 Mrads, and fed in diets to rats, resulted in almost a cessation in recycling of feces or coprophagy, whereas in the administration of 3 Mrads-treated beef diet this effect was not pronounced (26). The effect of coprophagy is, of course, of less importance than the primary cause of the hemorrhagenic syndrome brought about by destruction of vitamin K in the diet. Vitamin A administration plays a role in the hemorrhagenicity of certain diets. The type of protein in the diet relates to acceleration of hypoprothrombinemia, and DL-methionine has been shown to have a sparing effect on vitamin K (27).

Ascorbic acid is the most sensitive of the water-soluble vitamins to radiation treatment, the effect most pronounced in pure dilute solution (28). There is some protective action in foods by other vitamins such as niacin. Of the three B vitamins, niacin, riboflavin, and thiamine, the latter vitamin is the most radiosensitive in certain irradiated foods. Just as in the case with vitamin C, riboflavin in pure solution is more labile than in foods, where vitamin C and other components have protective effects. Niacin is radioresistant in aqueous solution, but in some foods where vitamin C is present the percentage of destruction of niacin increases rapidly upon irradiation. In a comparison of processing methods, it has been noted that thiamine destruction in several thermal-processed or canned foods is greater than that for radiation-sterilized products.

In assessing the probable pharmacological effects of continuous administration of irradiated foods to man or animals, in view of the foregoing comments relevant to radiation effects on the macro- and micronutrients, it must be remembered that a mixed spectrum of foods, raw and processed, would be more than adequate to prevent the development of specific physiological aberrations in man. In addition, vitamin losses may be obviated by supplementation of diet, if indicated or required. The vitamin K radiation damage and hemorrhagenicity observed in experimental animals, of course, has no relevance to man, since human dietaries are composed of high vitamin K-containing components in raw leafy green vegetables and other foods (29).

EVALUATION OF POSSIBLE TOXICITY

Microbiological Safety

Clostridium botulinum.—The greatest potential hazard of bacterial origin in radiation-sterilized foods is the possible occurrence of *Clostridium botulinum*, the causative agent of botulism. *C. botulinum* spores and their toxin are more radiation-resistant than any other pathogenic microorganisms which are apt to be present in foods. If the spores survive the irradiation treatment, prolonged storage of the food at high temperatures may favor germination and toxin production, thus creating an extremely hazardous situation. In such cases, the normal spoilage flora may have been partially or totally destroyed and no warning off-flavors, off-odors, or swelling of packaged food may be detectable. The remaining possibility of toxin destruction by thorough cooking of the food before consumption is not a completely reliable safety factor, since certain foods are frequently eaten in the raw or uncooked state.

To assure the complete destruction of these highly pathogenic, toxin-producing spores in radiation-sterilized foods, an inactivation factor of 10^{12} (12 D values) has been arbitrarily proposed among food microbiologists working in this area (30). The minimum radiation sterilization dose which fulfills this criterion for *C. botulinum*, type A (the most radiation-resistant of the six known types), is approximately 4.5 Mrads. Adherence to this concept is in general accordance with the equivalent thermal process inactivation for this organism, which has been practiced in the canning industry for many years.

The other known types of *C. botulinum* (types B, C, D, E, and F) may also present a similar hazard in various types of irradiated foods; however, all of these types are less radiation-resistant than type A. In the low-dose pasteurization treatment of marine products, type E spores are of particular interest, because of their presence in many marine environments and their ability to survive the rather low radiation dose ranges (0.1–0.4 Mrad) which appear to be most technologically feasible for extensions of storage life at refrigeration temperatures. Certain strains of type E spores are capable of outgrowth and toxin production at temperatures as low as 38° F (31). There appears to be a considerable delay in the outgrowth of type E spores surviving irradiation in marine products, as compared to that of unirradiated spores (32). The relation of time requirements for toxin production to consumer rejection due to organoleptic unacceptability of irradiated marine products held at specific temperatures within the refrigeration zone is extremely important.

Certain advantages have been found for combination processes of irradiation, followed by heat treatment for food sterilization. The irradiation sensitizes the *C. botulinum* spores to heat, thus requiring less severe treatment of either type to effect sterilization (33).

Salmonellae.—Salmonellosis has become recognized as a serious public health problem in many countries during recent years. The importance of animal and human food as transmitting agents of *Salmonella* organisms is well known. Poultry and poultry products are the sources of infection most frequently incriminated in food-borne outbreaks (34, 35). Frozen meats and dry proteinaceous animal feeds, such as meat, fish, and cottonseed meal, are often heavily contaminated with salmonellae and contribute greatly to the overall problem.

The salmonellae have been found to be the most radiation-sensitive of all pathogenic organisms in foods (36). Radiation doses required for complete inactivation of this group of organisms vary somewhat, depending on the serotypes present, environmental factors (e.g., atmosphere, temperature), and food substrate, but in general, an adequate dose for most foods has been found to be between 0.5 to 0.7 Mrad.

A panel of experts was convened by the International Atomic Energy Agency in December 1962, to discuss the potential role of radiation in control of *Salmonella* organisms transmitted by food and feed products (37). Radiation treatment of certain infected products appears to be a promising alternative, and in some cases the most practical method of freeing such products of salmonellae and similar organisms. The radiation resistance of *Salmonella* organisms, like other vegetative bacteria, is related to the state of water in the food. A radiation dose of 0.2 Mrad is extremely effective in lowering *Salmonella* numbers in frozen meats, poultry, and eggs with negligible effects on organoleptic qualities (4, 38).

Control of viruses.—The possible control of food-borne viruses and rickettsia with ionizing radiation has received rather meager attention to date. Studies have disclosed a rather remarkable radiation resistance of viruses in general (39). An inverse relationship between inactivation dose and size of virus has been noted (40, 41). The rate of inactivation is an exponential function of the radiation dose applied. A dose of 3.6 Mrads gamma radiation was found effective for inactivation of the Lansing polio, St. Louis encephalitis, and Western equine encephalomyelitis viruses when an initial population of one million LD_{50} were used (41). The vaccinia virus was somewhat less radiation-resistant. Irradiation by doses less than 1 Mrad has shown considerable promise for the preparation of noninfective diagnostic antigens (42).

The D_{10} value of certain viruses has been shown by several workers to approximate 1 Mrad (43, 44). The estimated 12 D dosage for human enteroviruses has been calculated to be approximately 10 Mrads (45). Dose requirements of such magnitude would obviate the possibility of irradiating foods for this purpose simply because of the adverse organoleptic changes which would be encountered. However, this calculated dosage may be more than double the actual practical requirement, since initial contaminating virus levels are not likely to occur in foods at such high levels and because viruses lack the ability to multiply in food during or after irradiation (46). In addition, most radiation-sterilized foods must be subjected to a preliminary light heat treatment to inactivate endogenous enzymes, and it is expected that some virus would be inactivated by this process.

Because of the relative radioresistance of viruses as compared to normal spoilage bacteria and their capabilities for reactivation and genetic recombination, it is possible that the exposure of virus-contaminated food to sublethal levels of irradiation may induce variant forms and increased rates of mutations (47). The potential importance to both human and animal disease of such possible effects of irradiation on food-borne viruses dictates the need for additional studies in this area.

The effects of ionizing radiations on the foot and mouth disease (FMD) virus have been investigated by Italian workers (48) with a viewpoint toward possible radiation sanitation treatment of contaminated animal products (e.g., meat, hair, hides, bones, glands). Inactivation of the FMD virus required approximately 3 Mrads in the liquid state and 4 Mrads in the dry state. Use of radiation for this purpose could have great importance in international commerce. There is a great need for further irradiation studies on FMD virus and other exotic animal viruses of economic importance, such as rinderpest, swine fever, and African swine fever.

Bacterial mutagens.—Multiple exposures of certain types of bacteria to sublethal doses of radiation, with alternate periods of vigorous growth, have been reported to result in the occurrence of mutant organisms which are significantly higher in radiation resistance than their parent generations (49–51). Morphological and physiological alterations of mutant organisms have been noted (52, 53). Changes were also observed in certain biochemical and serological properties related to identification of the organisms, while virulence and toxigenicity of *Salmonella* were decreased. Negative findings have been reported by other workers who exposed various strains of saprophytic organisms to repeated irradiations (54).

While the findings in this area are of considerable scientific interest, their significance to the public health safety of substerilization food processes has not been clearly established. It is known that heat treatment is capable of inducing high numbers of mutations in bacterial spores (55). There is no evidence of increased virulence in the pathogenic organisms tested or of acquisition of pathogenicity in nonpathogenic organisms as a result of repeated exposures to radiation. The possibilities of closely related organisms receiving multiple exposure in a practical processing situation appear rather remote. Nevertheless, it is conceivable that the development of radiation-resistant mutations could decrease the effectiveness of radiation processing of foods over a period of time and cognizance should be taken of future findings in this area.

Inactivation of Bacillus anthracis.—Since human anthrax often results from exposure to animal by-products which are contaminated with *Bacillus anthracis* spores, consideration should be given to the possible advantages of irradiation control of this health hazard. A minimum dose of 2 Mrads of gamma radiation has been successfully used on an industrial scale in Australia for the inactivation of *B. anthracis* in baled goat hair (56).

Parasite Control

The feasibility of low-dose irradiation control of trichinosis (*Trichinella spiralis*) has been aptly demonstrated (57–60). Doses of 12 to 18 Krads of ^{60}Co gamma rays, although not lethal to the trichinae larvae, were capable of breaking the life cycle by sterilizing the resultant adult females and rendering them incapable of reproduction or further development in the human intestine after ingestion. Calculations of probable costs (utilizing ^{60}Co or ^{137}Cs sources) indicated feasibility for commercial application in pork processing plants (61). No alterations in nutritive value or organoleptic attributes of pork are caused by this low-dose treatment. Theoretically, the irradiation of all pork carcasses would offer an absolute solution for the eradication and control of human trichinellosis in any country where there is a high incidence of infection. However, the rapid decline of incidence in the United States, for example, during the past 25 years (due to factors such as legal enforcement of cooking procedures for garbage fed to hogs, low temperature storage of pork, and public awareness of proper methods of cooking pork) would tend to discourage the possibilities of this application (62). The sporadic outbreaks which still occur in the United States are frequently traceable to animals raised for private slaughter and use, a situation which does not lend itself to effective controls other than proper cooking. However, efforts toward the complete eradication of trichinosis should continue and radiation treatment may prove to be more economical for this purpose than the presently used low temperature methods.

The possible control of the beef tapeworm, *Cysticercus bovis*, by irradiation of beef carcasses has been explored (57). As in the case of *Trichinella spiralis*, radiation doses up to 100 Krads were required to destroy the cysts *in situ*; however, exposures as low as 10 Krads will render the immature stages incapable of development when transferred to suitable hosts. This effect would appear to be of extreme value in a control program, since man is the reservoir of infection and the human form of this parasite (*Taenia saginata*) is transmitted via human sewage to cattle. Low-dose radiation treatment of beef carcasses for prevention of normal growth and reproduction of *C. bovis* would appear to be an effective and economical means of control for any country where the incidence of infection is high.

Insect Pest Control

There are two principal techniques for the radiation control of certain insect pests, which are of economic importance in food production. One possibility is the direct irradiation of the food products which contain them to destroy the insects or to induce permanent reproductive sterilization. This method appears to offer great potentialities and is best suited to the disinfestation of products handled in bulk, such as cereal grains. One drawback in bulk foods is the lack of protection against reinfestation which could negate the advantages of irradiation treatment.

The second method of radiation control of insect pest species is by the release of large numbers of sterile adults into the natural environment, and the competitive mating by wild and sterilized males over a number of generations to decrease gradually the wild population. Criteria for the success of this method include the availability of a mass rearing technique to produce the vast numbers of insects required, the single mating of the female during its life span, and the ability to sterilize adequately the insects without adversely affecting their motility, mating behavior, or longevity (63). Costs may be prohibitive because of the large numbers of insects which must be reared and the difficulty of preventing entry of fertile wild insects into the environment. This technique may have the greatest potential in rather limited or closed environments.

Fungal Control

Moderate range doses of ionizing radiation have been used for the destruction of certain types of storage fungi in grain and seeds. Cathode ray doses in the range of 0.23 to 0.38 Mrad were found to be lethal to spores of seven species of *Aspergillus* (64). Lethal doses of gamma radiation for suspensions of spores and mycelia of phytopathogenic fungi on agar were found to vary from 0.182 to 0.91 Mrad. The fungistatic effect appears to be dependent on both total dose and dose rate (65).

Although gamma radiation was found to cause some destruction of *Aspergillus flavus* and *Aspergillus amstelodami* spores which were inoculated into various crop seeds, the seedling production potential was reduced (65). Germination of wheat has also been found to be reduced by radiation doses as low as 50 Krads; therefore, on the basis of the limited work to date, it has been concluded that irradiation does not appear to offer a practical method for destruction of storage fungi of seeds intended for planting purposes (65). The possible radiation destruction and control of certain fungal species, such as the highly toxic group of mycotoxins which contaminate certain plant protein products, would appear to merit further investigation.

Chemical Additives and Degradation Products

Nonenzymatic browning.—Irradiation may produce degradation compounds which cause foods to undergo browning, sometimes referred to as the "Maillard reaction." Individual carbohydrates and amino acids may give rise to labile carbonylic compounds, which can either undergo browning reactions themselves, or react with amino compounds already present in the food or produced by the irradiation (66). Greater amounts of carbonylic compounds are formed in the muscle portion than in the fat of irradiated meat (67). The carbonyls may not exert much direct effect on odor or flavor qualities of meat, but some of them can react with odorous sulfydryl compounds or amines produced by irradiation and thus may cause indirect effects on organoleptic properties.

Lipid oxidations.—An important type of chemical alteration which occurs in many irradiated foods is the reaction of lipids and fats with atmospheric oxygen. In high-dose radiation sterilization, certain peroxides may be formed which can be reduced by exclusion of oxygen and use of low temperatures. Decomposition of the peroxides which are initially formed usually results in the production of substances with reactive carbonyl groups, such as aldehydes and ketones (20, 68). The carbonyl compounds are known to destroy some fat-soluble vitamins, expecially vitamin E, and may act as antagonists to others. Such compounds may reduce the digestibility of fats and interfere with intestinal absorption of certain fatty acids from the intestine.

Although these peroxidative changes are similar to those which occur from autoxidation through heating, the effects of irradiation appear to be more severe in certain respects. Carbonyl compounds have been observed in irradiated food even when air is excluded, with the necessary oxygen presumably derived from the ester groups of the glyceride molecules (69). The high concentration of free radicals produced by irradiation causes the numbers of oxidation chains to be greater and of shorter length, thereby resulting in less effectiveness of added chemical antioxidants (66).

The fat-soluble vitamins (A, E, and K) have been found to be more sensitive to ionizing radiation than are the majority of the water-soluble vitamins, probably because of their antioxidant properties. Under practical situations, the destruction of these vitamins is not considered to be a limiting factor. Under experimental conditions, male rats fed vitamin K-deficient diets containing radiation-sterilized beef and pork developed hemorrhagic diathesis (70,71). Vitamin K-deficient diets containing oxidized oils or nonirradiated beef or pork caused similar symptoms and deaths in rats. The relevance of these findings to human feeding of irradiated foods is highly questionable, since normal diets would contain a mixture of irradiated and nonirradiated foods, many of which contain high amounts of vitamin K.

Packaging Constituents

Since the objective of food irradiation in most cases is to reduce the number of viable microorganisms, precautions must be taken to protect the treated food against subsequent recontamination. A reasonable method of achieving such protection is to seal the food within a suitable container immediately before radiation treatment. Special attention must be given to the physical attributes of materials intended for the packaging of radiation-sterilized foods where long-term storage without refrigeration may demand more "rugged" materials, such as rigid metal containers with appropriate protective interior enamels or flexible laminate containers (72). The complete chemical composition of the packaging material must be known. Radiation-induced changes in any of the packaging material constituents may impart malodors or flavors to the food or may result in a migration of potentially toxic extractive products to the food. The nature and concentration of chemical extractives must be determined for each specific material under consideration to assure its safety. Extraction techniques, followed by infrared spectroscopy examination of residues for identification of individual components, have provided convincing safety evidence for a number of films which may be used for gamma-irradiated prepackaged foods in which the dose does not exceed 1 Mrad (73, 74). Various classes of polymeric materials for flexible packaging of foods to be irradiated up to 6 Mrads have also been successfully developed and tested.

SPECIAL PROBLEMS FOR BIOLOGICAL ASSESSMENT

Irradiation preservation of foods, specifically sterilization for extended shelf life and storage stability, requires that spoilage microorganisms, pathogens, and certain proteolytic enzymes be inactivated or destroyed. Enzymes which are natural molecular protein constituents of plant and animal tissues, if permitted to be present after the death of plant or animal tissue cells, may act catalytically during autolysis, releasing compounds such as amino acids and other breakdown products which impart undesirable flavors and physical textures to food products (75). Thermal processing of meats, for example, inactivates proteolytic enzymes and frozen foods are enzyme-inactivated prior to storage. Cathepsins, which are proteolytic enzymes, hydrolyze tissue proteins and these enzymes, not necessarily inactivated by radiation sterilization, must be inactivated by a precooking procedure prior to radiation processing (76, 77). Thus far, no alternate procedure has been developed to obviate these combined procedures, especially the precooking, which may contribute undesirable cooked flavors and odors. Other complementary procedures for protein denaturation and enzyme inhibition have been proposed, such as the use of chemical antagonists and ultrasonic and radio waves, but these have not been investigated.

Off-flavors induced by radiation changes in meat proteins are most pronounced in beef, less so in lamb and veal, and least in pork and chicken and some fishery products, which are the most resistant (78). These chemical changes, which occur on autolysis where enzyme inactivation is not resorted to, are accentuated also at high radiation doses such as 4.5 Mrads, which have been required to insure safety in terms of destruction of spores of *C. botulinum*. If enzyme inactivation is employed, therefore, and is additive to the high radiation dose changes, this compounds the problem of off-flavor and textural changes.

Another problem that has demanded extensive investigation is that of insuring the nonpresence of induced radioactivity. Among American and European investigators, presented with the same data on radionuclide formation, there have been some divergent views on acceptability of energy levels of the radiation source to be employed in radiation processing (79–81). Sources of radioactivity which directly or indirectly produce neutrons of any energy levels, are capable of producing radioactive elements and thus must be excluded in radiation exposure of foods. ^{60}Co and ^{137}Cs sources produce relatively low energy gamma rays (1.17 and 1.33 MeV) either directly or by conversion of beta particles by bremstrahlung into X rays. Induced radioactivity has not been found experimentally in foods exposed to ^{60}Co sources. Elements that are known to show isomer activation at the above listed energy levels are not present in food, or exist in such small traces that their activity cannot be detected.

If high energy levels are used, isomer activation may result, leading to measurable levels of induced radioactivity. In general, with reference to the occurrence of radionuclides induced by processing, the safety depends on the amount of radioactivity, half-lives of radionuclides formed, the time elapsed in storage of product prior to assay for detectable radioactivity, and the sensitivity of the radiation detection equipment.

The binding energy of the nucleus of an element determines the extent of production of photoneutrons. Accordingly, enough energy must be transmitted by the photon to the nucleus to permit escape of neutrons. Hence, photons with energies higher than 2.2 MeV will induce radioactivation. Deuterium, with a threshold of 2.2 MeV, beryllium at 6 MeV of energy, and most of the remainder of elements in periodic table having levels of 8 to 10 MeV of energy, will produce photoneutrons by overcoming the binding nuclear forces. At these energies there is small probability of such an event occurring because of the extremely small capture cross section. In experimental work, it has been noted that energy levels of 16 to 18 MeV may produce detectable amounts of radioactive atoms.

No detectable induction of activity has been found in foods irradiated at energy levels below 10 MeV, with radiation doses of 4.5 to 5.6 Mrads (82). It would appear, therefore, that the energy threshold for the (γn) reaction in food elements exceeds 10 MeV. Some radioactivity would appear in foods treated at 13 MeV, involving the following elements: N-13, Na-22, Na-24, P-33, Fe-55, Zn-65, RB-84, I-126, and CS-132. The only long half-lives are Fe-55 and Na-22, whereas others range from a few minutes to 245 days for Zn-65. The element primarily responsible for radioactivity in food treated at high energy levels is Na-22. Electron energy levels as high as 24 MeV, used experimentally in radiation treatment of foods, are produced by electron accelerators and X radiation.

The radioactivity produced is vanishingly small at the low million electron volt range, and there is indeed some uncertainty with respect to the biological effects at very low doses and low-dose rates. It is believed, therefore, that there is a threshold below which no biological effect may occur (83). The induced radioactivity produced experimentally in the process herein considered must be viewed also in the context of the omnipresent naturally occurring radionuclides K-40, C-14, and Ra-226. Despite the considerations that naturally occurring radionuclides are present, there is a prevailing body of opinion that no induced radioactivity can be tolerated unless there is a balancing of benefit against risk. In essence, justification of the exposure of man to radionuclides induced in food by radiation sterilization is somewhat tenuous. Acceptance or clearance of an irradiated food should therefore rest on proof of biological harmlessness of the exposure and on the commercial advantages of such products. Currently, the added risk to the general population does not justify the irradiation of foods at energy levels beyond 12 MeV.

Another problem in assessment of the wholesomeness of irradiated foods is the concept of radiomimetic effects or "stored energy" of irradiated components, which was first advanced in 1960 (84, 85). In essence, "stored energy" is the change of energy of a system occurring when it is irradiated. The energy released is believed to act in a manner similar to radiation and thus, in a biological system, is able to act in a manner comparable to direct irradiation of cells or the total organism. Barley, potatoes, and fruit juices that were irradiated at doses of 20 to 200 Krads were investigated for their potential of radiomimetic effects. By observing effects on chromosome aberrations in plant tissue and changes in leukocyte counts in rats fed irradiated foods or injected with irradiated glucose solutions, certain cytological disturbances, such as chromosome breakage, were reported (86). The radicals formed may be hydroperoxides (87), which may produce effects comparable to ethylene oxide, methylmethane sulfonate, busulfan (Myleran), or other compounds. For example, when rats were fed irradiated bread treated at 3 to 6 Mrads there was a decrease in lymphocyte count (86).

The biological implications of these earlier findings on "stored energy" are quite tenuous in that the work was preliminary and not extensive enough to ascertain whether such formed radicals and the inherent "stored energy" in certain classes of food components would disappear after storage of irradiated foods. These observations have been based on only a few experiments on foods immediately after irradiation. The alteration in lymphocyte count may be of doubtful significance, since this effect may be confounded by a variety of environmental factors.

Extensive long-term or short-term feeding tests, conducted with various animal species and man in the United States, have not revealed any toxicity or histopathologic changes. While one may conclude from these large scale studies that there were no harmful changes, the biological testing techniques may have lacked sufficient sophistication to elucidate cytological effects and chromosomal aberrations. It is maintained, however, that these formed radicals and reaction products dissipate on storage, that only dry foods tend to retain this radiomimetic effect, and that all foods, on heating, will lose the stored energy, especially under the influence of moisture or high humidity.

In chronic toxicity investigations on irradiated foods, extensive evaluation has been made as to potential carcinogenicity. The preceding discussion on radiomimetic effects leaves an inference as to mutagenicity and carcinogenicity. The controlled human feeding studies that have been conducted are of such limited duration (15 days) that no adequate appraisal can be made in man of the absence of chronic toxicity or carcinogenesis. Accordingly, the probable absence of such effects must be based on extrapolation from experimental animal data. Well-designed animal feeding trials permit extrapolation to be made so as to allow for differences in the susceptibility of various species, and also in the differences between the numerical size of the experimental group and the human population.

A wide spectrum of irradiated foods and food components has been tested for carcinogenic activity, using rats, mice, and dogs as test species. Several techniques of applying these irradiated materials to experimental animals have been used, including long-term feeding, injection of and painting on skin of food extracts. No statistically significant differences in rate of tumor formation have been observed with either rats or mice challenged with irradiated and nonirradiated foods (19). In the radiation process, peroxides, epoxides, lactones, and quinones may be formed in food as a result of oxidative degradation. Since compounds in this class are potentially mutagenic and carcinogenic, it is essential that additional information on extent of formation of such substances during irradiation or storage be obtained; additionally, the most recent and sophisticated bioassay techniques should be employed to ascertain critically the absence of any mutagenic-carcinogenic activity.

Paralleling the earlier reports on "stored energy" or potential radiomimetic effects has been the recent concern over biological effects of radiation, which are mediated indirectly by radiolysis products of sugar. Recent investigations have demonstrated a marked inhibitory effect on cell division of carrot cells grown on irradiated media, or media supplemented with irradiated sugar solution, when high radiation doses are used (0.5–4.0 Mrads) (88). An opposite effect or stimulatory action on cell division is noted at lower doses (0.02–0.5 Mrad). Gene mutations were observed on *Drosophila* raised on media containing irradiated glucose, which is comparable to chromosome aberrations produced in meiotic and mitotic cells of plants raised on media supplemented with irradiated sucrose. This evidence for increase in mutations can be considered suggestive of an effect, but the relevance of this phenomena to mammalian systems may have little significance. Radiation products of irradiated sucrose, for example, may be rendered ineffective from a mutagenic standpoint by enzymatic degradation in the gut or by detoxification processes in mammalian target organs. Metabolic tracer studies in mammalian systems should prove the validity of this concept. Heat processing of foods produces compounds similar to those in radiation treatment, and this aspect of the problem may have interesting comparative findings.

GENERAL PROBLEM IN ASSESSMENT OF POTENTIAL TOXICITY AND EXPERIMENTAL DESIGN

Acute and Chronic Toxicity Protocols

The formation of potentially toxic and carcinogenic compounds in irradiated foods has been recognized as a distinct possibility. In the United States, the 1958 Food Additives Amendment to the Food, Drug and Cosmetics Act (89) has included the radiation treatment of foods with other processing methods which are subject to critical appraisals concerning possible toxicological and carcinogenic hazards.

In evaluating the safety of a conventional food additive, the specific chemical which is to be added is normally well characterized and its interactions with the normal food components can often be predicted. Such knowledge is extremely helpful, since it enables the researcher to focus attention on the more important effects of such interactions. The extremely complicated, often poorly elucidated chemical changes which occur in foods as a result of irradiation have made it nearly impossible to specify any particular compounds which might conceivably give rise to hazards in higher mammals and man. Other conditions which may complicate the chemical alterations in irradiated food include the composition of the food, environmental conditions during irradiation, total radiation dosage and dose rate, postirradiation storage conditions, and the introduction of combined processes (e.g., heat, chemical additives), domestic cooking, and other procedures (3).

In evaluating the safety of radiation-processed foods, the factors of nutritional adequacy and toxicity must be considered and studied independently as far as possible. Comprehensive reviews have been written on this subject (19). Subacute and chronic animal feeding experiment protocols have been established to obtain data of this nature on many types of irradiated foods. The subacute tests have involved the feeding of control and irradiated foods to animals at a level of approximately 35 per cent of the total dietary solids, for periods of approximately 90 days. These tests are presumed to represent a significant challenge for eliciting any potential abnormal physiological response. The chronic study (usually of two years duration) is an extension of the short-term study with a larger animal population (with additional species of animals in many cases). In the case of rats or chickens, observations are made over a period of four generations. Both chronic and subacute experiments are designed to yield information concerning growth, food efficiency, hematology, enzyme and urine studies, gross pathology, and histopathology (carcinogenicity). The chronic studies also provide information on reproduction, lactational performance, and longevity.

Detailed requirements concerning animals to be used in toxicity and carcinogenicity tests have been reviewed and outlined by the Expert Committee on the Technical Basis for Legislation of Irradiated Food which met in Rome in April 1964 (3). Particular attention has been stressed in the selection of animals (species, strain, sex, and numbers) for studies of this nature. Experiments must be designed properly in order to provide ample information upon which realistic decisions can be based. All data must be analyzed by acceptable statistical procedures. Where doubt arises concerning a negative response in an experiment, or where a high margin of reliability in data is lacking, larger numbers of animals and additional experiments may be necessary in order to estimate the carcinogenic hazard (90).

Despite the fact that the experimental animals are provided with diets of known nutritional requirements for adequate growth and development, the high level of test food which is incorporated in the diets may represent a completely unrealistic situation which can place a nutritional stress on the animals and result in nutritional imbalances. An example of this situation has been observed in the feeding of high levels of irradiated egg solids to dogs where the interrelationship between biotin and avidin was found to exert a role in causing reproductive failure. A related example of difficulty which has been experienced in separating potential toxicity and nutritional adequacy of irradiated foods was the previously mentioned effect of radiation sterilization on vitamin K (antihemorrhagic factor) in certain foods, which resulted in hemorrhage and death in chicks and rats. Careful and detailed studies are necessary to elucidate the mechanisms involved in physiological abnormalities of this nature.

Extrapolation of Toxicological Data on Experimental Animals to Man

Since man is the ultimate consumer of irradiated foods, human experiments on irradiated foods would provide the most ideal evidence of safety. Short-term clinical studies on human beings consuming irradiated foods have been conducted (19). Since it is not feasible to obtain human volunteers for studies of any significant duration, considerable reliance for toxicity and carcinogenic data must be placed on the results of comprehensive animal tests. An adequate margin of safety must be incorporated in order to validly translate animal data into an assessment of the potential human hazard. In the case of food additives, a margin of safety of aproximately 100 has been used (91). This margin of safety is desirable to compensate for such factors as species difference in susceptibility, numerical differences between experimental animals and human population under exposure, and the relative homogeneity of animals as compared to man. It can be predicted with a reasonable degree of assurance that the absence of a toxic or carcinogenic effect in well-designed animal feeding studies will indicate the probable absence of an effect in man. Whenever possible, however, well-controlled human clinical studies, even of short duration, would be desirable to add confidence to the extrapolation of animal data to man.

Extrapolation from Existing Wholesomeness Data on Irradiated Foods

In certain cases,-it may be possible to extrapolate from wholesomeness data which have previously been compiled and critically evaluated. If the results of complete long-term chronic toxicity studies on at least two species of animals (one rodent and one nonrodent species) have clearly established the absence of toxicity for a representative food of a class, the necessity of performing similar studies on foods closely related in origin and chemical composition may be obviated. Short-term, subacute toxicity studies (described above) or specified chemical analyses on such related foods may be adequate to establish their safety. The results of short-term studies which are performed on this basis must be carefully evaluated. If no evidence of toxicity is detected in animals at the conclusion of the 90 day study, it is generally assumed that no further testing of the food is necessary. However, if any type of adverse biological effects have been detected, the full scale (long-term) study would be mandatory for that particular food before a decision on its safety for consumption could be made.

There is a general viewpoint that extrapolation of experimental data on a particular food which has been irradiated at specified dose levels may be considered for the same food item treated with lower radiation doses. There has been reluctance toward extrapolation of data in the opposite direction, e.g., from low to higher dose levels. Sufficient evidence concerning lack of adverse chemical changes and effects on major nutrients due to the lowered dose level(s) is often required when extrapolations of this nature are made.

The vast amount of data which has been accumulated on numerous types of irradiated foods has presented no evidence of radiation-induced toxicity or carcinogenic effects (92, 93). However, judgments concerning requirements for toxicological experiments on irradiated foods must continue to be exercised on an individual proposal basis after careful consideration of all evidence.

REQUIREMENTS FOR NATIONAL AND INTERNATIONAL HEALTH LEGISLATIVE CONTROL AND METHODS FOR IDENTIFICATION OF IRRADIATED FOODS

It has been recommended by several international committees that the use of ionizing radiation for food processing be under public health and legislative control (3). Any specific irradiated food or spectrum of technologically acceptable irradiated foods available for distribution and public consumption should be evaluated as to safety for consumption on the basis of evidence from a battery of tests relating to nutritional value and innocuity of the product with reference to toxicity (91). In certain countries, particularly the United States of America, the radiation treatment of food as a process comes within the scope of legislation designed to control food additives (89). In other countries, such as Germany, special regulations governing the use of ionizing radiations for treatment of food have been promulgated.

In the context of legislative and public health controls, foodstuffs treated by ionizing radiation must be defined and the technological purpose of such a treatment must be provided. With respect to the treatment, a complete description of the irradiation equipment, nature of the rays and energy they release, the characteristics of the emitting source, conveyor facilities detailing position of food with respect to source, and the dwell time of food within the radiation field must be specified. In addition, description of precautions to insure the safety of plant workers who will irradiate food must be provided, as well as identification of equipment to eliminate contamination risks.

In the description of a radiation process, the maximum and minimum radiation dosage must be specified. Storage conditions, including packaging and containers for food, temperature of storage, and any time limit within which the foodstuffs must be consumed or utilized should be delineated. In order to define adequately the product or identify radiation-processed foodstuffs which are subject to legislative controls, certain procedures have been proposed for this purpose.

Sterility control is important to establish that viable microorganisms have been eliminated from the irradiated food and continual surveillance of the process must be maintained. Radiation-sensitive tapes, which change color on exposure to radiation, have been affixed to packages of surgical supplies or sutures for monitoring such packages by plant operators to establish a sterilization process. Similarly, such a procedure could be used on irradiated food packets or containers. For biological testing, the introduction of packs in the processing line, such packs inoculated with suitable nonpathogenic organisms, can be used for subsequent testing of the containers or packages to establish that these organisms have been destroyed by the radiation treatment.

Food products may contain bacterial toxins before irradiation. If contaminated with toxigenic bacteria, the radiation treatment must insure that no toxins are formed if such spore formers as *C. botulinum* are present. Radiation processing differs from heat treatment since *C. botulinum* is readily destroyed by heat. Testing experimental batches of food for safety from pathogenic toxins is time-consuming and costly. There would thus be considerable advantage if a nonpathogenic organism or marker could be used to approximate the same resistance as C. *botulinum*. There has only been one such organism proposed, which is *Micrococcus radiodurans*. This organism simulates *C. botulinum*, since it possesses high radiation resistance (94). Assurance for microbiological safety and control must be based on good sanitation in operations, incubation tests for swollen container detection, adequate radiation dose to prevent toxin formation, control of storage temperatures in radiation-pasteurized food (low-dose radiation—shelf life extension), and proven safety of packaging for prevention of recontamination.

Establishing the adequacy of nutrient content in radiation-processed foods does not pose such a problem as that of microbiological safety. The effect of ionizing radiation on food components other than the vitamins is generally quite small. The vitamin losses, as have been previously discussed, are comparable to those observed in thermal processing. It is quite unlikely that the nutrient loss will be significant in relation to overall human dietary effects and the subsequent influence on human nutrition. In certain countries where irradiated foods may conceivably comprise a major portion of the dietary, special consideration would have to be given to a particular food in relation to the nutrient value of the national diet as a whole. Special situations such as diets of infants, elderly and debilitated persons exhibiting restriction on selection and variety, might influence the total nutrient level in utilization of irradiated foods. Government controls, supported by nutritional surveys, would be indicated to guarantee that no special or vulnerable groups in the population could be adversely affected in this regard.

Since radiation treatment of foods represents an innovation in preservation procedures, it is essential that physical and biological criteria be used bv governmental public health authorities to identify such foods and that suitable declarations be made on the containers or packages that such foods are processed by ionizing radiation. Such labeling would not only help in public health control per se, but would be of value to the consumer in providing instructions for handling and storage. Previous reference has been made to the fact that colored labels affixed to the package may reflect that a certain dose of radiation has been absorbed. These labels, attached to the package prior to irradiation, would also indicate and avoid confusion as to whether the food contained therein was irradiated or nonirradiated.

LITERATURE CITED

1. Wüst, O., French Patent 701, 302, 1930, *Chem. Abstr.*, **25**, 4068 (1931)
2. Brasch, A., and Huber, W., *Science*, **105**, 112–17 (1947)
3. FAO/WHO/IAEA, Report on the Technical Basis for Legislation on Irradiated Food, *Food Agr. Organ.*, Atomic Energy Ser., No. 6 (1965)
4. Ingram, M., and Roberts, T. A., *IAEA/FAO Intern. Symp. Food Irrad., Karlsruhe, Germany 1966*, (In press)
5. Goldblith, S. A., *J. Am. Dietet. Assoc.*, **31**, 243–49 (1955)
6. Kraybill, H. F., Read, M. S., Linder, R. O., Harding, R. S., and Isaac, G. J., *J. Allergy*, **30**, No. 4, 342–51 (1959)
7. Johnson, B. C., *FAO-IAEA Report of the Brussels Meeting on the Wholesomeness of Irradiated Foods*, 61–74 (1962)
8. Burks, R. E., Baker, E. B., Clark, P., Esslinger, J., and Lacey, J. C., *J. Agr. Food Chem.*, **7**, 778–82 (1959)
9. Merritt, C., Bresnick, S. R., Bazinet, M. L., Walsh, J. T., and Angeline, P., *J. Agr. Food Chem.*, **7**, 784–87 (1959)
10. Wick, E. L., Yamanishi, T., Wertheim, L. C., Hoff, J. E., Proctor, B. E., and Goldblith, S. A., *J. Agr. Food Chem.*, **9**, 289–93 (1961)
11. Tsien, W. S., and Johnson, B. C., *J. Nutr.*, **68**, 419–28 (1959)
12. Partmann, W., *FAO-IAEA Report of Brussels Meeting on the Wholesomeness of Irradiated Foods*, 75–96 (1962)
13. Newman, A. A., *Food Manuf.*, **33**, 374–378, 422–25 (1958)
14. Herisset, A., *Compt. Rend.*, **239**, 1438–39 (1954)
15. Bernheim, F., Wilbur, K. M., and Kenaston, C. B., *Arch. Biochim. Biophys.*, **38**, 177–84 (1952)
16. Swanson, M. A., and Artom, C., *J. Biol. Chem.*, **187**, 281–87 (1950)
17. Haurowitz, F., Schwerin, P., and Yenson, M. M., *J. Biol. Chem.*, **140**, 353–59 (1941)
18. Andrews, J. S., Mead, J. F., and Griffith, W. H., *Federation Proc.*, **15**, 918–20 (1956)
19. Kraybill, H. F., *Intern. J. Appl. Radiation Isotopes*, **6**, 233–54 (1959)
20. Monty, K. J., Tappel, A. L., and Groninger, H. S., *J. Agr. Food Chem.*, **9**, 55–58 (1961)
21. La Fontaine A., Deschreider, A., Bugyaki, L., *FAO-IAEA Report of the Brussels Meeting on the Wholesomeness of Irradiated Foods*, 97–112 (1962)
22. Natarajan, A. T., Nordic Meeting on Food Preservation by Ionizing

Radiation, Danish Atomic Energy Commission, *Risö Rept.*, No. 16, 39–40 (1960)

23. Ehrenberg, L., and Von Ehrenstein, G., Nordic Meeting on Food Preservation by Ionizing Radiation, Danish Atomic Energy Commission, *Risö Rept.*, No. 16, 41–43 (1960)
24. DaCosta, E., and Levenson, S. M., *U. S. Army Med. Nutr. Lab. Rept. 89* (1951)
25. Mellete, S. J., and Leone, L., *Federation Proc.*, **19**, 1045–49 (1960)
26. Metta, V. C., Mameesh, M. S., and Johnson, B. C., *J. Nutr.*, **69**, 18–22 (1959)
27. Reber, E. F., and Malhotra, O. P., *Proc. Contractors' Meeting, 7th, Chicago*, QMC Radiation Preserv. of Foods Project, 147–50 (1961)
28. Proctor, B. E., and Goldblith, S. A., *Nucleonics*, **5**, 56–62 (1949)
29. Kraybill, H. F., *FAO-IAEA Report of the Brussels Meeting on the Wholesomeness of Irradiated Foods*, 44–47 (1962)
30. Schmidt, C. F., *FAO/IAMS Meeting on the Microbiology of Irradiated Foods, Paris, Rome* (1961)
31. Schmidt, C. F., Lechowich, R. V., and Folinazzo, J. F., *J. Food Sci.*, **26**, 626–30 (1961)
32. Segner, W. O., *Intern. Symp. Food Irrad., Karlsruhe, Germany, 1966* (In press)
33. Kempe, L. L., and Graikoski, J. T., *Appl. Microbiol.*, **10**, 31–36 (1962)
34. Quist, K. D., *Public Health Rept. (U. S.)*, **78**, 1071–73 (1963)
35. Wilder, A. N., and MacCready, R. A., *New Engl. J. Med.*, **274** (26), 1453–60 (1966)
36. Thatcher, F. S., *Can. J. Public Health*, **55**, 151–57 (1964)
37. IAEA Report on Radiation Control of Salmonellae in Food and Feed Products, *IAEA Techn. Rept. Ser.*, No. 22 (1963)
38. Mossel, D. A. A., and Groot, A. P. de, *Radiation Preservation of Foods*, 233–64 (NAS-NRC, Washington, D. C., 1965)
39. Pollard, E. C., *Advan. Virus Res.*, **2**, 109–51 (1954)
40. Desrosier, N. W., and Rosenstock, H. M., *Radiation Technology in Food, Agriculture and Biology* (AVI Publ. Co., Westport, Conn., 1960)
41. Jordan, R. T., and Kempe, L. L., *Proc. Soc. Exptl. Biol. Med.*, **91**, 212–15 (1956)
42. Polley, J. R., *Can. J. Microbiol.*, **7**, 135–39 (1961)
43. Wilson, D. E., *Radiation Res.*, **14**, 796–802 (1961)
44. Pollard, E. C., *Ann. N. Y. Acad. Sci.*, **83**, 654–56 (1960)
45. Kaplan, H. S., and Moses, L. E., *Science*, **145**, 21–25 (1964)
46. Cliver, D. O., *Radiation Preservation of Foods*, 269–74 (NAS-NRC, Washington, D. C., 1965)
47. Thatcher, F. S., *Gamma Radiation Can.*, **2**, 17–24 (1961)
48. Massa, D., Application of Food Irradiation in Developing Countries, *FAO/IAEA Techn. Rept. Ser.*, No. 54, 159–71 (1966)
49. Erdman, I. E., Thatcher, F. S., and MacQueen, K. F., *Can. J. Microbiol.*, **7**, 207–15 (1961)
50. Kelner, A., Bellamy, W. D., Stapleton, G. E., and Zelle, M. R., *Bacteriol. Rev.*, **19**, 22–44 (1955)
51. Witkin, E., *Genetics*, **32**, 221–48 (1947)
52. Idziak, E. S., and Thatcher, F. S., *Can. J. Microbiol.*, **10**, 683–97 (1964)
53. Pontefract, R. D., and Thatcher, F. S., *Can. J. Microbiol.*, **11**, 271–78 (1965)
54. Pepper, R. E., Buffa, M. T., and Chandler, V. L., *Appl. Microbiol.*, **4**, 149–52 (1956)
55. Chiaison, L. P., and Zamenhof, S., *Can. J. Microbiol.*, 12, 43–46 (1966)
56. Horne, T., Turner, G. C., and Willis, A. T., *Nature*, **183**, 475–76 (1959)
57. Taylor, E. L., and Parfitt, J. W., *Intern. J. Appl. Radiation Isotopes*, **6**, 194–98 (1959)
58. Gomberg, H. J., and Gould, S. E., *Science*, **118**, 75–77 (1953)
59. Gould, S. E., Van Dyke, J. G., and Gomberg, H. J., *Am. J. Pathol.*, **29**, 323–37 (1953)
60. Gibbs, H. C., MacQueen, K. F., and Pullin, J. W., *Gamma Irradiation Can.*, **2**, 29–37 (1961)
61. Gomberg, H. J., Gould, S. E., Nehemias, J. V., and Brownell, L. E., *Nucleonics*, **12** (5), 38–46 (1954)
62. Most, M. J., *J. Am. Med. Assoc.*, **193** (11), 871–73 (1965)
63. Knipling, E. F., *J. Econ. Entomol.*, **48**, 459–62 (1955)
64. Bridges, A. E., Olive, J. P., and Chandler, V. L., *Appl. Microbiol.*, **4**, 147–49 (1956)
65. Golumbic, C., *Intern. Symp. Food Irrad., Karlsruhe, Germany, 1966* (In press)

66. Lea, C. H., *Intern. J. Appl. Radiation Isotopes*, **6**, 86–94 (1959)
67. Batzer, O. F., Sribney, M., Doty, D. M., and Schweigert, B. S., *J. Agr. Food Chem.*, **5**, 700–3 (1957)
68. Monty, K. J., *Federation Proc.*, **19**, 1034–37 (1960)
69. Chipault, J. R., Privett, O. S., Mizuno, G. R., Nickell, E. C., and Lundberg, W. O., *Ind. Eng. Chem.*, **49**, 1713–20 (1957)
70. Johnson, B. C., Mameesh, M. S., Meta, V. C., and Rama Rao, P. B., *Federation Proc.*, **19**, 1038–44 (1960)
71. Read, M. S., *J. Agr. Food Chem.*, **8**, 346–49 (1960)
72. Tripp, G. E., *JCAE Hearings 87th Cong., 2nd Session*, 942–47 (U.S. Govt. Printing Office, Washington, D.C., March 1962)
73. Payne, G. O., and Spiegl, C. J., *Radiation Preservation of Foods*, 99–107 (NAS-NRC Publ. 1273, Washington, D.C., 1965)
74. U.S. Code Fed. Regs. Food Additives Title 21, Food and Drugs Ch. 1, Subch. B, Part 121, Subpart F, Sec. 121.2543 (Aug. 10, 1964)
75. Proctor, B. E., *Proc. Intern. Conf. Preserv. Foods Ionizing Radiation, Cambridge, Mass., 1959* (MIT Publ. 74–90, July 1959)
76. Barron, E. S. G., Dickman, S. R., Singer, T. P., and Muntz, J. A., *Biological Effects of External X and Gamma Radiation*, Chap. 15 (E. Zirkle, Ed., McGraw-Hill, New York, 1954)
77. Sui, R. G. H., Ed., *Radiation Preservation of Food*, 169–76 (Office of U.S. Army Q.M. Corps, U.S. Govt. Printing Office, Washington, D.C., Aug. 1957)
78. Doty, D. M., and Wachter, J. P., *J. Agr. Food Chem.*, **3**, 61–63 (1955)
79. Strauch, K., *Ann. Rev. Nucl. Sci.*, **2**, 505–28 (1953)
80. Glass, R. A., Smith, H. D., and Newkirk, W. H., *Trans. Am. Nucl. Soc.*, **1**, Session 23, 254–56 (1960)
81. Leveque, P., *FAO-IAEA Report of The Brussels Meeting on the Wholesomeness of Irradiated Foods*, 157–65 (1962)
82. Smith, H. D. (Stanford Res. Inst., Army Q.M. Final Contr. Rept., March 1962)
83. Federal Radiation Council Report No. 2 (May 1960)
84. Westermark, T., Nordic Meeting on Food Preservation by Ionizing Radiation, Danish Atomic Energy Commission, *Risö Rept.*, No. 16, 28–36 (1960)
85. Natarajan, A. T., and Swaminathan, M. S., *Ind. J. Genet. Plant Breeding*, **18**, 220–23 (1958)
86. Ehrenberg, L., *FAO-IAEA Report of the Brussels Meeting on the Wholesomeness of Irradiated Foods*, 166–78 (1962)
87. Scholes, G., and Weiss, J., *Radiation Res.*, Suppl. 1, 177–89 (1954)
88. Holsten, R. D., Surgii, M., Steward, F. C., *Nature*, **208**, 850–56 (1965)
89. U.S. Congress, 85th Session, Public Law 85-929, Food Additives, Amendment to Federal Food, Drug and Cosmetics Act (6 Sept. 1958)
90. Shubik, P., and Sice, J., *Cancer Res.*, **16**, 728–42 (1956)
91. FAO/WHO Rept. Expert Comm. Food Additives, *World Health Organ., Techn. Rept. Ser.*, No. 144 (1958)
92. Kraybill, H. F., *Intern. Conf. Radiation Preserv. Foods, Boston, Mass., 1964* (NAS-NRC Rept. 1273)
93. Raica, N., and Howie, D. R., *IAEA/FAO Intern. Symp. Food Irrad., Karlsruhe, Germany, 1966* (Rev. U.S. Army Wholesomeness Irrad. Foods Program, 1955–1966) (In press)
94. Anderson, A. W., Nordon, H. C., and Carn, R. F., *Food Technol.*, **10**, 575–77 (1956)

CONTENTS