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An evaluation of the utilization of animals exposed to lethal levels of ionizing radiaiton

Gerhard R. Eisele

To the Graduate Council:

I am submitting herewith a dissertation written by Gerhard R. Eisele entitled "An evaluation of the utilization of animals exposed to lethal levels of ionizing radiaiton." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

M. C. Bell, Major Professor

We have read this dissertation and recommend its acceptance:

R. L. Murphree, R. R. Shrode, M. R. Johnston, R. L. Tugwell

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

March 3, 1971

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and recommend its acceptance:

R. L. Murphree

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Vice Chancellor for
Graduate Studies and Research

AN EVALUATION OF THE UTILIZATION OF ANIMALS EXPOSED TO LETHAL LEVELS OF IONIZING RADIATION

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

G. R. Eisele

March 1972

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ABSTRACT

Animals exhibiting visible signs of radiation sickness could be an important food resource in the event of a nuclear disaster. Market hogs (Sus scrofa domestica) weighing approximately 200-250 lbs were exposed to a total air dose of 700 R at a rate of 1 R or 45 R/min of gamma radiation from a ^{60}Co source. Bacteriological studies were conducted on pre- and post-irradiation blood specimens. Muscle, liver and mesenteric lymph nodes were cultured for bacteria 10 days post-irradiation when the animals were slaughtered, and muscle samples were cultured again 5 days post-slaughter during which time the carcasses had hung in a cooler. Samples of the teres major muscle were taken at the above times for the following chemical analyses: 1) crude protein, 2) soluble protein, 3) ether extract, 4) moisture content and 5) pH. Cooked loins from carcasses of irradiated and control animals were subjected to sensory panel evaluations.

A bacteremic state was not detected in the 1 R/min group nor was there a greater incidence of bacterial isolates from the carcasses of irradiated animals as compared to those from the control animals. Staphylococcus aureus was one of several organisms recovered from the meat. Since several strains are capable of producing an enterotoxin, the safety of the meat for consumption is questionable since no tests are available to rapidly identify these strains. The results of determination of chemical properties indicated no significant differences in ether extract, moisture content, crude protein or soluble protein. The pH of the meat from irradiated animals at slaughter was significantly

higher ($P < .05$) than that of control meat. During the storage phase the pH decreased significantly ($P < .01$). Taste panel evaluations indicated no drastic flavor differences due to treatment. However, color was significantly affected.

All animals exposed to 45 R/min developed a bacteremia at least once during the 10-day post-irradiation period, but this bacteremia was not consistent within the group. Bacterial isolates from the muscles of irradiated animals at slaughter and 5 days later were consistently higher than isolates from controls. These bacteria represented an increased number of genera. Because of the possibility of non-specific and staphylococcal food poisoning, it is recommended that this meat not be consumed. Muscle from irradiated animals yielded significantly more ether extract at slaughter ($P < .05$) and after 5 days storage, while moisture content was significantly lower ($P < .05$) at both times. A significant reduction ($P < .01$) in soluble protein was noted during the storage phase. Muscle from irradiated animals again yielded significantly higher pH values ($P < .05$), but no difference was noted after the storage phase. Taste panel evaluations indicated less treatment effect on both flavor and color than observed in the low dose rate group.

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CHAPTER I

INTRODUCTION

Throughout history, the propagation and consumption of food has been of utmost importance for the survival of mankind. One of the results of the machine age and its progeny, the atomic age, is the increasing dependency of man on his complex society. Modern man is primarily concerned with those factors of production in which he is involved. Only when a natural or man-made disaster occurs and food supplies become scarce, does man realize the great extent of his dependency. In a postattack situation one of the major requirements for human survival will be safe and adequate availability of food supplies. Maximum utilization of livestock will help tremendously in satisfying this requirement. The USDA Radiological Training Manual (1961) indicates that animals which do not show visible signs of radiation sickness may be used safely for human consumption. The NAS-NRC subcommittee (1963) concluded that animals could be salvaged for food if slaughtered within 2 to 8 days after exposure or when they have completely recovered from the radiation sickness syndrome, i.e., no evidence of illness or elevated temperatures. However, in a postattack situation, these guidelines may not be feasible, particularly in hard-hit areas. Animals showing visible signs of radiation sickness might be an important source of food as well as a nucleus to reestablish breeding herds or to be utilized at a later date.

There is an inconsistency in the literature on bacterial invasion into the circulatory system of radiation-sick animals, and little work

with large animals has been reported. Therefore, studies were initiated to determine if exposure to lethal doses of radiation adversely affects the value of livestock salvaged for human food. This subject was subdivided into categories which might provide answers to the following questions: 1) Do farm animals develop bacteremia? 2) Does further invasion into the organs and muscles take place? 3) Does radiation alter the normal chemical changes in muscles? 4) Is the meat from these animals safely consumable under postattack conditions?

CHAPTER II

LITERATURE REVIEW

Infection has been considered an important factor in the death of animals which received whole-body ionizing radiation. The effects of infection and the defense mechanisms of the animals are quite complex. Susceptibility to infection is caused chiefly by a reduction in the number of neutrophiles (neutropenia) which results from damage to the radiosensitive precursor cells of the hematopoietic tissues, as well as damage to other defense mechanisms. These defense mechanisms include the immune processes which are impaired to various degrees after whole-body irradiation, viz., phagocytic function, bactericidal action of serum and mechanical barriers (i.e., skin, internal mucosae of the respiratory and alimentary tracts) against entry of microorganisms.

For more information pertaining to the above subject material, the reader is directed to the books by Bond, Fliedner and Archambeau (1965), by Casarett (1968) and by Rubin and Casarett (1968) and the volume edited by Hollaender (1955).

Miller, Hammond and Tompkins (1951) exposed white male mice weighing approximately 20 g to 450 or 600 R whole-body x-radiation at a rate of approximately 20 R/min. In the 450-R group, 595 mice were terminated and selected tissues were cultured. From this group of 595 mice, 152 had positive bacterial cultures from the spleen and/or heart blood, with 66% of the cultures occurring during the second week post-irradiation. In the 600-R group, 288 mice were terminated, and these yielded 113 positive cultures from the heart's blood and/or spleen.

Of these mice, 70% showed more than 50 colonies per drop of heart's blood. The period of greatest mortality was in the second week when 80% of the positive cultures were found. These authors concluded that a majority of the animals had a bacteremia severe enough to be considered an overwhelming sepsis and, to a large degree, responsible for their deaths. This bacteremia was caused by microorganisms normally present in the lower bowel. In both the 450-R and 600-R groups, 91% of the cultures were pure (mostly gram-negative rods commonly found in the intestinal tract) and the remaining 9% contained only two microorganisms. The authors noted that the bacteremia rose and fell during the second post-irradiation week, roughly parallel with the daily death incidence. Hatch et al. (1952) conducted similar studies, and similar results were reported by Hammond (1963) when mice were fed Pseudomonas aeruginosa.

Warren and Whipple (1923) irradiated dogs with doses of X-ray (radiation exposures not given) that destroyed a large amount of the epithelial covering of the crypts and villi of the small intestine. The animals were terminated 1, 2, 3 and 4 days after exposure. After the second and third day, the intestinal epithelium showed severe injury, disintegration and separation from the basement membrane. Four days post-irradiation there was evidence of invasion of bacteria into the bloodstream and various organs. They concluded that no overwhelming invasion of the tissues, lymph, and blood by intestinal bacteria had occurred, indicating that perhaps the intestinal epithelium is not the all-important barrier which protects the tissue from intestinal invasion of bacteria. Chrom (1935) reported that exposing the entire abdomen, liver and spleen of mice gave rise to progressive bacteremias and that

the epithelial cells of the intestine may lose the ability to retain the bacteria normally present in the intestinal lumen. Similar results were reported by Gordon, Cooper and Miller (1955).

Lawrence and Tennant (1937) studied the comparative effects of filtered 200-KV X-rays and neutrons on 6- to 8-week-old Swiss mice. The X-ray dose was 700, 800, 900 and 1,000 R at 35 R/min, and the neutrons were produced in a cyclotron by bombarding beryllium with 5-MV deuterons at 10 μA with neutron doses (in roentgens) of 223, 245 and 298 R. The viscera and blood were sterile in mice killed 1, 2, 3 or 4 days after 1,000 R X-radiation. However, some animals that died during the first week post-irradiation did yield positive cultures. As the dose was decreased and the life span increased, positive cultures were first isolated from the viscera and then from the heart's blood. Most of those which died 5 to 11 days after irradiation with 700 to 800 R gave positive cultures. These authors concluded that the organisms (usually Bacillus coli) entered the circulatory system through the damaged mucosa of the intestine. They concluded that the mechanism of death from both forms of radiation was a combination of tissue destruction and enterogenous infection, the former of these predominating in the acute deaths. Therefore, as the dose is decreased and the animal lives longer, bacteremia is a usual finding and infection an important factor as to the cause of death.

Schechmeister and Bond (1951) exposed NAMRU strain of mice (5 to 6 weeks old) to 350 R of X-radiation at 250 KV at a dose rate of 25 R/min. Six days later the irradiated and control mice were inoculated subcutaneously with either live or heat-killed Pasteurella pestis (avirulent

strain A-1122) and live Escherichia coli. Irradiated mice inoculated with either live or heat-killed avirulent P. pestis and live E. coli had a higher mortality than control mice receiving the same treatment. When live avirulent bacteria were injected, the organisms recovered were the same or autogenous bacteria. When heat-killed organisms were inoculated, the organisms reisolated were of autogenous origin only. These authors concluded that death was associated with bacteremia.

Kaplan, Speck and Jawetz (1952) reported that homozygous strain C57 black mice were exposed to between 450 and 485 R of whole-body X-radiation at 32 R/min and challenged 1 to 3 days post-irradiation with beta-hemolytic streptococci. The infection spread more rapidly in the irradiated infected animals than in the infected controls. The cumulative mortality curves in the two groups were parallel, but the mean survival time was significantly reduced in the irradiated group by approximately 4 days. This suggests that the bacteremia, once developed, differs only in the rate of development. Similar results were observed by Hammond, Anderle and Miller (1959), who concluded that the susceptibility to infection was related to the rate of irradiation, rather than to the accumulated dose.

Mice which received 485 R 2 days before infection showed a greater number of positive tissue cultures than their respective infected controls. Heart blood at 2 days post-infection showed heavy growth in all cultures. Similar results were observed from the muscle, lymph node, lung, liver and spleen (Kaplan, Speck and Jawetz, 1952).

Furth, Coulter and Howland (1952) exposed 24 adult dogs to 450 R of whole-body X-radiation at a rate of 7.15 R/min. Twelve of these dogs received aureomycin at the rate of 100 mg/kg per 24 hours for 28 days

post-irradiation. The remaining 12 animals served as controls. The incidence of positive blood cultures was 8.5% greater in the irradiated control group. The number of positive blood cultures in the control group increased, while the number of positive cultures decreased in the treated group. The number of coliform organisms in the feces increased in both groups, reaching a maximum during the second week; however, the fecal coliform bacteria were higher in the group receiving aureomycin. These bacteriologic studies of the feces showed a shift in bowel flora with an increase in the staphylococci and coliform bacilli. These authors concluded that the observed changes were related to the control of infections and infectious processes by aureomycin. Similar results were reported by Freter (1966).

Osborne et al. (1952) exposed C57 Black Jax mice to whole- and partial-body X-radiation in what the authors termed "acute intestinal" radiation death and typical radiation death doses. The dose range and lethality for the above are approximately 1,200 to 1,500 R (LD = 100%) and up to about 600 R (LD = up to 70%), respectively. When mice were exposed to 3,000 R and terminated 1.5 to 3.5 days later, there was some evidence that a bacteremia had developed (one of four on 2 days post-irradiation and one of two on 3.5 days post-irradiation developed a bacteremia). The authors concluded that this is characteristic of acute intestinal radiation death in which a bacteremia is nonexistent or occurs just before death and is of no critical importance.

Partial irradiation of the head and thorax only, abdomen only or LD-50 whole-body exposures led to a high incidence of bacteremia. Mice which survived 10 days were then sacrificed and the heart, spleen and liver were examined for bacteria. Irradiated mice with shielded

abdomens also developed bacteremia which led these authors to conclude that radiation damage of the intestine was not a necessary factor in post-irradiation bacteremia; and, since irradiation of the head and neck is sufficient to cause death, the bacteria may enter through the oropharynx. These findings indicate that bacteremia is a major factor in typical radiation death (Osborne et al. 1952).

Gonshery, Marston and Smith (1953) exposed 935 male mice of the NIH strain to X-radiation with doses of 550, 625, 700, 800, 1,100 and 1,400 R at a dose rate of 56 to 62 R/min. Bacteriological studies were conducted on blood, spleen and heart blood. One group exposed to 625 R was given daily doses of 5 mg of streptomycin sulfate (injected subcutaneously) from the 3rd through the 28th day post-irradiation. These authors reported that at the higher doses of 1,000 and 1,400 R, more than half of the mice showed no evidence of infection in either blood or spleen and that positive blood cultures were associated with survival times. Most of the bloodstream infections were noted after 3.5 days, but at the highest dose rate the animals did not live long enough for septicemia to develop. However, they did find that 36 of the spleens and 46 of the blood samples yielded organisms.

Of the mice exposed to 700 and 800 R, 65% showed organisms in the heart blood. In the 550-R and 625-R groups, certain infections did reduce the survival time and were associated with Pseudomonas or E. coli infections and/or streptococci. These authors concluded that infection was responsible for a number of deaths which would not have occurred otherwise among mice given these lower radiation doses. It was noted also that the presence of bacteria in blood or spleen at death did not necessarily mean that death was caused by infection or that the absence

of bacteria at death is not proof that infection was not involved in the animals' deaths. Smith, Gonshery and Marston (1953) reported similar results but with a higher percentage of positive blood cultures.

Shechmeister and Adler (1954) exposed 5- to 6-week-old, white, female strain R mice to X-radiation doses of 250 and 350 R at 200 KV at a dose rate of 21 R/min. This strain R had repeated severe spontaneous outbreaks of pseudotuberculosis, and the effect of sublethal irradiation as an activator was studied. Nearly all the dead animals had pseudotuberculous lesions in one or several organs from which Corynebacterium pseudotuberculosis was isolated. The lungs, spleen, lymph nodes, kidneys and liver were most frequently involved. These authors concluded that X-radiation activated a latent or subclinical pseudotuberculosis in these animals and that their exposure to a sublethal X-radiation led to an epidemic. It was noted also that a clinical pseudotuberculosis was transmitted by both direct and indirect contact to mice of a different strain (NAMRU) and source which were irradiated also, but not to those animals which were not irradiated.

Pillemer (1955) reported on the properdin system, stating that it is a natural defense mechanism of the blood which destroys certain bacteria, neutralizes some viruses and lyses certain abnormal erythrocytes in the absence of specific antibodies. When the serum properdin level is altered, it appears to influence the course of infections and the susceptibility of laboratory animals to the effects of whole-body irradiation.

After lethal levels of whole-body irradiation of rats and mice, the serum properdin dropped rapidly to less than 30% of normal levels. Pillemer concluded that properdin is an important part of the defense

mechanism by reporting that mice became very susceptible to infection with an E. coli strain which could be an important factor in the development of bacteremias.

Kiselev, Nikitina and Shao-chang (1965) administered intraperitoneal injections of E. coli endotoxin to guinea pigs for 10 days in total doses of one-twentieth to one-sixtieth of the LD-50 dose. These authors concluded that with prolonged administration of the endotoxins a hemorrhagic syndrome developed which was characteristic of radiation sickness and coincided with the appearance of specific antiendotoxins in the blood. They felt that the disappearance of the hemorrhagic syndrome and the formation of specific antibodies indicated that autoinfection plays an important part in the initiation and development of the hemorrhagic syndrome in acute radiation sickness.

Bradner, Bernstein and McCarthy (1955) exposed an inbred BUC strain of mice to a total dose of 600 R at 200 KV at a dose rate of 240 R/min. Serological comparisons were made of bacteria isolated from the blood, heart, spleen and feces to determine if the infections originated from the gut. This was done by comparing a given species of organisms isolated from the blood and tissues with that isolated from the feces. Three species of organisms were studied, Proteus mirabilis, Paracolonbactrum coliforme and Pseudomonas aeruginosa. These authors concluded that the organisms found in the blood and organs of the X-irradiated mice were serologically identical to bacteria of the same species found in the feces and the animals' own gastrointestinal tracts are the source of these organisms. Mottram and Kingsbury (1924) reported similar findings. Bradner and Hald (1955) conducted a similar experiment where Proteus mirabilis was given in drinking water of mice for 5 days

before irradiation, and the mice were then placed on a basal diet with oxytetracycline for 2, 5 and 10 days post-irradiation. The authors reported that the maximum number of bacteremias occurred on the 11th day, the first being observed on the 8th day. Similar results were reported by Burrows, Deupree and Moore (1950).

Mayhew et al. (1955) investigated the invasion of microorganisms through the intestinal wall in female burros, Equus asinus asinus. These animals were exposed to whole-body gamma radiation at a rate of 40 R/hr, a total of 25, 100 and 400 R/day till death. Microorganisms commonly found in the intestinal tract of the experimental animals also were isolated from the blood and internal organs after irradiation at 25 R/day. This would suggest that a bacteremia did develop in these animals.

In the animals receiving 100 R/day, the organism Serratia marcescens was inoculated into the cecum through an artificial fistula before and during the period of irradiation. Then 10 ml of the cecal contents were withdrawn and 10 ml of a 5.0×10^9 cells/ml suspension were introduced by means of a pipette. The Serratia marcescens organism was found in the blood and internal organs and in vitro was able to traverse the intestinal wall of irradiated burros but not that of the nonirradiated burros.

Mayhew et al. (1955) concluded also that in vivo and in vitro studies of the 400-R/day burros indicated that the intestinal wall had not changed sufficiently to permit microorganisms to pass through before death at 9 days post-irradiation.

Gordon et al. (1955) irradiated 11- to 12-week-old Rockland RAP female mice with a single dose of 700-R whole-body X-radiation at

approximately 28 R/min. The following tissues and/or organs were cultured for bacteria: 1) mesenteric lymph nodes, 2) mesenteric lymph, 3) liver, 4) portal blood, 5) heart's blood, 6) spleen, and 7) cervical lymph nodes. The test organism Serratia marcescens was established in the intestinal tract by administering it in the drinking water. The organism was recovered from the mesenteric lymph nodes of almost half of the normal mice examined, indicating that bacteria in small numbers are able to pass through the nonirradiated gut to the regional lymph glands.

After irradiation the liver and spleen became infected with enteric organisms before the bloodstream was invaded, indicating that the reticuloendothelial system was able, for a time, to deter a bacteremia even after the immediate defense barriers from the intestinal tract were broken down. The greatest incidence of positive portal and heart's blood occurred in the periods 8 to 9 days and 8 to 11 days, respectively, with the earliest detection being 4 to 5 days post-irradiation for either type of blood.

Boone, Woodward and Harris (1956) studied the incidence of bacteremia with mortality to determine the time of appearance of bacteria in heart blood and various tissues following thermal column and X-ray exposures, as well as doses given in the so-called "acute intestinal death" range. The X-ray exposures of adult CF1 mice were given at dose rate of 247 R/min. The thermal column exposures were given at a dose rate of 1.37 rem/sec.

Dose ranges of 400 to 800 R of X-ray and 480 to 616 rem of thermal column radiation showed that all mice which died the first 11 days post-irradiation had a bacteremia which contributed to the deaths of the

animals. The highest incidence of positive tissue and heart blood cultures occurred during the period surrounding the median survival time. The mesenteric lymph nodes were the first tissues to become positive, then the spleen and liver tissues and, lastly, the heart blood. At doses of 765 rem and 950 R, positive heart blood cultures were not found for the first 3 days, although tissue cultures were positive. The mean survival time was 4 to 6 days. With doses of 640 to 950 R of X-ray and 480 to 765 rem of thermal column exposure, the percentage of positive blood and tissue was greater during the median survival times. As the dose was decreased, a greater time lapse between exposure and appearance of positive cultures was noted. After 3,000 R of X-ray or 3,000 rem of thermal column radiation, heart blood was essentially negative for the 3 days before death; however, 40 to 80 of the mesenteric lymph nodes and some of the spleen cultures were positive by the third day. At 10,000 R some of the tissues and heart blood cultures were positive by the first day and continued to be so until death. Similar results were also reported by Edmondson and Batchelor (1965) when they subjected British Saamen goats to doses ranging from 200 to 2,400 rads of fission neutrons, or 200 to 2,000 R of gamma rays at a dose rate of 2,500 rad/hr and 1,950 R/hr, respectively. These authors concluded that although few deaths could be attributed to infection and though blood cultures were sterile, bacterial aggregates were nearly always found in the tissues, most commonly in the intestines, liver and lungs. They noted that fever occurred invariably in the few days before death.

Brown et al. (1961) exposed Hereford cattle 3 to 5 years of age to lethal levels of whole-body gamma radiation. These authors noted an

increase in rectal temperature at the end of the irradiation period in 80% of these irradiated animals. At approximately 14 days post-irradiation, rectal temperatures again increased with an average survival time of 5 days after the onset of fever.

Silverman et al. (1957) conducted bacteriological studies in CF1 male mice exposed to supralethal doses of ionizing radiation from a nuclear device as well as subsequent experiments using fast neutrons and X-radiation from laboratory sources (Silverman et al., 1958). In the first experiment mice exposed to neutrons or combined neutron and gamma radiation from a nuclear device became moribund within 2 to 4.5 days. Of the neutron irradiated group, 78% gave positive blood and/or spleen cultures, and 96% of those receiving the combined radiation effect showed positive cultures. In all cases, the organisms isolated were bacteria normally found in the intestinal tract. In addition, two or more organisms were isolated from the majority of the animals. The spleen was the organ most frequently infected, indicating that this organ is still capable of filtering bacteria but incapable of destroying them since a bacteremia eventually develops.

In the later experiment, death occurred within 5 days and the rate of infections paralleled the increase in number of deaths. During the first 60 hours, bacteria were isolated from the spleen and then from the bowel. This confirms their earlier conclusions that post-irradiation infection played a minor role in the deaths of animals exposed to supralethal doses of ionizing radiation. A similar conclusion was reported by Bond, Silverman and Cronkite (1954) and by Vogel et al. (1954).

Noyes, Evans and Baker (1963) reported on swine that were exposed to a nuclear detonation (Operation Plumb-Bob) which resulted in an appreciable number of wounds and burns. The animals were simultaneously subjected to gamma plus neutron irradiation of 1,034, 615 and 268 reps which resulted in a substantial number of wounds and burns. Of the 235 blood cultures taken 4 and 6 days post-detonation from living animals (varying in distance from the detonation), 45 were positive. The micro-organisms most commonly found were Staphyloccoci, P. multocida, beta-hemolytic Streptococci and Corynebacterium species (in that order of frequency). Heart blood at necropsy showed 56 positive cultures from all animals, 31 of these having multiple species. Staphylococci occurred more frequently in the blood of animals dying during the first 9 days after the blast. Wounds were highly populated by a polymicrobic flora, clostridia being isolated quite frequently. The bacterial flora in the wounds decreased after the third day. These authors concluded that some of the organisms arose from sources other than the gastro-intestinal tract and suggested that a microbic invasion influenced by a nuclear weapon effect had occurred. Similar results were reported by Reid et al. (1955) who also concluded that the site of the tissue injury and local flora therein appears to determine the type of invading bacteria.

Wasserman and Trum (1955) in a unique short-term experiment reported that the flesh from lethally irradiated animals (cows and sheep) was not detrimental when ingested by canines. Two cows were exposed to 6,400 R and 700 R of continuous gamma radiation from a ^{60}Co source at approximately 400 R/hr and terminated. One of the sheep was exposed to 3,600 R and died 90 hours post-irradiation; the other sheep was exposed to 5,280 R and died 132 hours post-irradiation. The meat was ground and frozen for later use.

The dogs were fed this meat for 128 to 129 days at which time they were terminated and autopsied. Deviations from "normal" weights of the lungs, heart, spleen, liver, kidney, brain, thyroid, and adrenals were statistically nonsignificant.

Prochazka et al. (1966) studied the incidence of bacteremia in direct relation to leucopenia and regeneration potency of the white blood cell picture in birds. Some 6- to 12-month-old New Hampshire cocks and hens were exposed to X-radiation doses ranging from 600 to 2,000 R at a rate of 40 R/min. The most frequent positive bacteriological findings in organs were obtained between the fourth and eighth days, i.e., in the same period during which deepest leucopenia occurred. These authors concluded that there was a correlation between the degree of damage due to leucopoiesis, particularly to its regeneration potency, and the incidence of bacteremia in irradiated birds. Similar results were reported by Bennett et al. (1949) in their work with dogs.

Pawel, Kalousova and Vranovska (1967) exposed sows and barrows to 700 R of gamma radiation (^{60}Co) at 3 R/min and slaughtered the animals either before, during or after recovery from the clinical symptoms of acute radiation sickness. Various organs and/or tissues were obtained for microbial evaluations after slaughter. The incidence of bacteria was greater in the groups of irradiated animals than in the control animals, i.e., from the liver, spleen, kidney, lymph nodes and muscle tissue. These authors concluded that penetration of bacteria outside the intestinal tract occurs before the clinical symptoms of acute irradiation sickness have developed. They concluded also that the bacteria are found first in the organs (particularly in the liver and spleen) and then in the

muscles. The majority of the microorganisms found consisted of coliform and streptococci.

Silaev (1962) exposed female rabbits weighing 2.5 to 3 kg to a total dose of 800 rads (length of exposure, about 8 minutes) to study the change in the carbohydrate-phosphorus metabolism in muscle tissue. Samples for analysis were taken from the longissimus dorsi muscle. The greatest change was observed 24 hours after irradiation with a considerable drop in the total carbohydrate content, due to a lower monosaccharide content. When the muscle was preserved at 3° to 5°C, a more rapid breakdown of glycogen occurred with a reduction in the monosaccharide content. In normal muscle an accumulation of monosaccharide occurs. Also, smaller amounts of lactic acid were found in the muscles of the irradiated group as compared to those of the control group.

Vranovska, Kalousova and Pawel (1966) exposed hogs to a total dose of 700 R (since the dose rate was not given, it is assumed to be the same as in Pawel et al. 1967 i.e., 3 R/min). These authors studied changes in pH, ammonia and water with the values determined under refrigeration and at room temperature. The hydrogen-ion concentration in meat from irradiated hogs was almost always higher than that in meat from control animals (during the duration of meat storage) and appeared hydremic during storage. Larger amounts of ammonia were found during storage in meat of irradiated animals which caused earlier onset of spoilage. These authors concluded that the ripening and spoilage process was faster in the meat from irradiated animals than in that from control animals. Also, the storage life of the meat was proportional to the degree of development of the radiation sickness syndrome.

Sukhomlinov, Datskiv and Khmil (1971) reported on the influence of X-rays on the protein component of myglobin which was isolated from muscles of dogs exposed to 600 R. The amino acid composition, topography and number of peptides of tryptic hydrolyzates, as well as the absorption curves in the infrared region of the spectrum of the proteins, were changed post-irradiation.

Further information pertaining to research efforts and interests in muscles and/or meats categories can be found in the textbook edited by Briskey, Cassens and Marsh (1970).

CHAPTER III

EXPERIMENTAL PROCEDURES

A. Animals

The experimental animals used in these studies were Duroc barrows obtained from the University of Tennessee-Atomic Energy Commission Agricultural Research Laboratory (UT-AEC ARL) swine herd. All animals were examined by a veterinarian for any abnormalities or disease prior to irradiation. Hematological evaluations were conducted pre-irradiation to evaluate further the animals' health. Some post-irradiation hematology was done but on a limited basis because of the development of hematomas and the possibility of artificially causing the development of a bacteremia. A total of 15 animals were randomly assigned to the low dose rate study. Eleven of these animals were exposed to 1 R/min and the remaining four animals served as controls. Seven animals randomly chosen, five irradiated and two control animals, were used for chemical analysis of the muscle. In the high dose rate study six animals were exposed to 45 R/min and two animals served as controls. Chemical analysis of the muscles from all of these animals was conducted.

In addition to the ear-notch identification, all animals were brand painted. At the time of irradiation, the barrows were approximately 8 months of age, weighing 200-250 lbs. The animals were confined pre- and post-irradiation in a restricted lot outdoors where they were given water and a 16% protein ration ad libitum. They had access to shelter at all times.

B. Irradiation

On the day of irradiation, the animals were transported to the irradiation facility, placed in restraining cages, and then taken into the facility for radiation exposure. The ^{60}Co Variable Dose Rate Irradiation Facility at UT-AEC ARL (Cheka et al., 1971) was used as the radiation source in these studies. A total bilateral exposure dose of 700 R (air dose) of gamma radiation at either 1 R/min or 45 R/min was given. After irradiation the animals were transported back to their pens and observed daily for signs of radiation sickness.

C. Blood Bacteriology

Blood samples were collected under sterile conditions and incubated to detect any signs of bacteria in the bloodstream (bacteremia). It was assumed that the presence of bacteria in the blood would indicate that the breakdown of the animals' defense barriers had occurred.

The following procedures were used for the collection of blood and subsequent bacterial evaluations. The animal was restrained and the entire neck area was washed with soap, then 95% alcohol and then tincture of Merthiolate. Clean gauze pads were used to apply each of these washing solutions. An 11-cm, 18-gauge, sterile bleeding needle was attached to a sterile syringe and the needle lightly flamed. The animal was bled from the carotid (carotis externa) artery or the jugular (jugularis externa) vein. Approximately 6 ml of blood were drawn from the animal and the needle withdrawn and flamed thoroughly. The needle was then removed, the syringe opening lightly flamed, and the blood inoculated into a culture medium (thioglycollate).

The culture medium tube opening was flamed before and after the blood was introduced into the medium and then sealed and marked. The culture medium was then incubated at 38°C for a maximum of 72 hours. If no growth was noted at the end of the incubation period, the sample was classified as negative. If growth was observed in the medium, a gram stain was made and secondary inoculations were made onto Blood Agar, Eosin-Methylene Blood Agar, Staphylococcus 110 Agar, Phenylethanol Blood Agar and MacConkey Agar. Bacterial colonies from these media were then incubated in various differential tube media which contained a fermentable carbohydrate.

D. Necropsy

The animals were weighed, then brought to the abattoir where conventional slaughtering procedures were then followed. The liver, lymph nodes (mesenteric) and various muscle tissues were evaluated for bacterial activity. The surface of the culture area was rendered aseptic either by the application of a red-hot spatula or by the application of direct flame. A flamed (red-hot) knife was then used to make a small puncture opening through which a sterile swab was thrust to absorb cellular fluid, removed and introduced into the culture medium (thioglycollate). The culture medium tube was flamed before and after introduction of the swab. Duplicate cultures were made from selected organs. Blocks of flamed tissues were introduced into culture medium after observing aseptic conditions in the medium. The culture medium was incubated for a maximum of 72 hours at 38°C. If no growth was observed at this time, the sample was classified as negative. If growth was observed, the same steps were taken as were taken with blood.

The hams and loins were removed, the hams cured in the normal process and the loins frozen for later use in a sensory panel evaluation. Bacterial evaluations also were carried out at these times. Teres major muscle samples were taken at slaughter for chemical analysis and again after the carcasses had hung in a cooler for 5 days. These samples were vacuum packed in 303X306 tin cans and frozen until a later date. The meat was then ground while still frozen, placed in small plastic bags and returned to the freezer for use the next day. The analysis consisted of determination of moisture, crude fat, crude protein, pH and soluble protein. These determinations were carried out as described by the AOAC (1960) with the modifications noted below.

Moisture Content

Approximately 3 g of the ground meat sample were used. After drying, this sample was then returned to the desiccator and used for the ether extract determination.

Crude Fat or Ether Extract

The sample previously dried for moisture determination was transferred to folded filter paper, placed in a stainless-steel thimble and inserted into the thimble holder of a Goldfisch apparatus. The sample was refluxed with 45 ml of anhydrous ether for 6 hours at a condensation rate of approximately 5 drops/sec.

Crude Protein

A premixed catalyst (Harshaw Scientific, Trade Name Kel-Pak No.5) was added to the Kjeldahl flask. Added to this were 40 ml of concentrated sulfuric acid.

pH

A Corning semi-micro combination electrode was inserted directly into a small hole which was made in the muscle. Approximately three random readings were recorded for each muscle. All pH readings were adjusted to the temperature of the muscle.

Soluble Protein

Total ninhydrin-positive material determination was used as an estimate of the total amino acids. This method, described by Tallon, Moore and Stein (1954), involves deproteinization, lyophilization, reaction with ninhydrin and measurement of optical density.

Lean-meat samples weighing 10 g each were blended with a ten-fold volume of 1% picric acid for 5 minutes in a Waring Blender. The picric acid blend was centrifuged for 10 minutes at 2,000 rpm. The supernatant was filtered with Whatman No. 1 filter paper, and the final protein-free extract was brought to a volume of 100 ml. Two 50-ml aliquots were transferred to Erlenmeyer flasks and immediately frozen with dry ice and acetone. The frozen samples were lyophilized, sealed and stored at -23°C until time for determination.

From the redissolved diluted sample, 0.1 ml was pipetted into a test tube, and 1 ml of ninhydrin reagent was added. The tube was capped, placed in boiling water for 20 minutes and the solution diluted with 5 ml of n-propanol-water diluent. The optical density of the heat solution was measured with a Bausch and Lomb Spectronic 20 at the wavelength of 570 μm. The millimoles of ninhydrin-positive materials were calculated from a standard curve prepared using leucine.

The leucine standard curve was prepared by the method of Moore and Stein (1948). Six different concentrations of leucine solutions ranging from 0.05 to 2.0 mM were prepared. A 0.1 ml standard was transferred to a test tube, and 1 ml of ninhydrin reagent was added. The tube was capped, placed in boiling water for 20 minutes and the solution diluted with 5 ml of n-proponal-water diluent. The optical density was measured with a Bausch and Lomb Spectronic 20 at the wavelength of 570 μm.

E. Sensory Evaluations

A triangle test was incorporated to detect any flavor and color difference of irradiated as compared to control meat. Each panel member received three coded samples, two of which were identical, and was asked to indicate which was the odd sample. All meat samples (loin) were handled, cooked and served as nearly alike as possible. Colored lights were used during the flavor evaluations to reduce the possibility of color as a criteria for selection. Color evaluations were conducted separately after the members finished with the flavor phase.

F. Rat Experiment

Female rats, 1 to 2 years old, weighing approximately 300 g were exposed to 800, 900 and 1,000 R whole-body gamma radiation at 50 R/min. Of the 30 animals used for each level of radiation, 5 were untreated, 10 irradiated and 15 irradiated and bled. Blood samples were taken under diethyl ether anaesthesia via cardiac puncture when the rats showed symptoms of radiation sickness or when the rats were in the moribund state. All blood samples were collected under aseptic conditions by the same procedure as previously described.

G. Statistical Analysis

A split-plot experimental design was the basis for this research, and the statistical analyses of the chemical variables were conducted accordingly. The animal-within-treatment mean square was used as the error term for testing the difference between observations from irradiated animals and those from control animals; and the animal x time-within-treatment mean square was used as the error term to test the effects of time x treatment, time (days) and animal within treatment.

CHAPTER IV

RESULTS AND DISCUSSION

A. Low Dose Rate Animals (1 R/min)

Results of the bacteriologic examination of the blood, muscle, liver and lymph nodes of swine exposed to a dose rate of 1 R/min, total dose 700 R, at the time of slaughter (10 days post-irradiation) and 5 days post-slaughter (carcass in cooler at $0^{o}C$) are tabulated in Tables Ia and Ib.

Blood samples evaluated for bacteremia were negative, and it appears that a bacteremia was not present in these swine, indicating that the reticuloendothelial system was not impaired to the extent that systemic bacterial invasion occurred under these conditions of irradiation.

In both the irradiated and control animals, very high percentages of positive cultures were obtained from the mesenteric lymph nodes 91 and 100%, respectively. Comparison of the two indicates a slightly higher number of genera isolated from the irradiated group. For the most part, the truly hemolytic (beta) streptococci are recognized as pathogenic, indicating that the alpha and gamma streptococci could be of a focal infectious nature. The function of the lymph nodes is twofold: 1) the production of lymphocytes and 2) the filtration of foreign material that comes from the lymph. It appears that these bacteria could have gained entrance from the intestinal lumina area and were retained in these lymph nodes. Microorganisms may cause inflammation at the site of entry or in the corresponding lymph nodes. It was concluded that the latter had

TABLE I

BACTERIA ISOLATED FROM CONTROL AND IRRADIATED SWINE SLAUGHTERED 10 DAYS AFTER EXPOSURE TO 700 R AT 1 R/MIN

Tissue	Positive Cultures (No./Total)	Positive Cultures (%)	Summary of Bacteria Isolated
a. Irradiated Swine			
Lymph Nodes (mesenteric)	10/11	91	α-hemolytic Streptococcus β-hemolytic Streptococcus λ-hemolytic Streptococcus Staphylococcus aureus Staphylococcus albus Bacillus subtilis Escherichia coli
Liver	3/11	27	β-hemolytic Streptococcus Staphylococcus aureus Bacillus subtilis Escherichia coli Aerobacter sp.
Muscle[a]	1/11	9	Staphylococcus aureus
Muscle[b]	2/11	18	Staphylococcus aureus Escherichia coli
Blood	0/11	0	-

TABLE I (continued)

Tissue	Positive Cultures (No./Total)	Positive Cultures (%)	Summary of Bacteria Isolated
b. Control Swine			
Lymph Nodes (mesenteric)	4/4	100	β-hemolytic Streptococcus Staphylococcus albus Bacillus subtilis Escherichia coli Aerobacter aerogenes
Liver	1/4	25	Escherichia coli
Muscle[a]	2/4	50	λ-hemolytic Streptococcus Staphylococcus aureus Staphylococcus albus Pseudomonas sp. Aerobacter aerogenes
Muscle[b]	1/4	25	Staphylococcus aureus
Blood	0/4	0	-

[a]Sampled at time of euthanasia.

[b]Sampled 5 days post-slaughter.

occurred in these animals. These results are in agreement with Gordon et al. (1955).

The number of different bacterial types isolated from the liver was greater in the irradiated animals than in the control animals (Tables Ia and Ib). The reduction of circulating leucocytes and overwhelming bacterial proliferation could have created a condition that permitted organisms to gain entrance into the portal circulation and be retained by the liver. Since the reticuloendothelial system was not impaired completely, it was thus still capable of maintaining the sterility of the blood. The above hypothetical sequence of events provides a plausible explanation of the negative blood culture. Similar results were reported by Bond, Silverman and Cronkite (1954), Gordon, Cooper and Miller (1955), and Pawel, Kalousova and Vranovska (1967).

Normal, healthy muscle tissue is generally bacteria-free, but insanitary implements or practices will contribute to contamination and spoilage of the carcass. One major source of bacterial contamination is the animal's hair and hide which could allow the bacteria to enter or penetrate the carcass and continue along moist surfaces or into the blood vessels, nerve sheaths and/or lymph vessels. The reason for chilling the carcass after slaughter is to prevent a rapid multiplication of microorganisms; bacterial growth is either prevented or impeded at these lower temperatures. The glycogen-lactic acid reaction which occurs in meat and changes the pH of muscles may produce an increase in temperature of the muscle hours after the carcass has been placed into a chilling room. This temperature increase may allow these organisms to continue to grow and increase in the area of contamination. Bacteria can cause a wide range of deteriorative changes including varying change of characteristic odor,

sourness and profound putrefaction, which is usually due to the breakdown of protein by anaerobic bacteria. Other bacteria will increase competitively and produce certain biochemical and physical changes which may be characterized by odor, taste, texture (such as softening of the tissue) and color changes due to the production of lactic, acetic or butyric acids, ammonia, trimethylamine, indol and sulfur compounds. Most of the food poisoning bacteria (Staphylococci, Salmonella, Shigella, Brucella) usually produce few outward changes when growing on meat, making odor or taste of questionable value in evaluating the safety of meat (Blanck, 1955 and Miller, 1958).

With respect to utilization of this muscle as food for human consumption, two categories or definitions can be made concerning the role of bacteria: 1) the multiplication of pathogenic organisms in the food itself (infectious type, such as Proteus vulgaris) and 2) following the ingestion of food in which poisonous substances have been formed due to the proliferation of the bacteria (toxic type, such as Staphylococcus aureus). In the infectious type, symptoms can occur from 4 to 36 hours after consumption, whereas in the toxic type, the incubation periods is in the range of 1 to 6 hours (Wilson and Miles, 1961). Staphylococcus aureus was isolated from muscles and could be one of these toxic types. Staphylococci are common inhabitants of the human skin, nasopharynx, etc. and may possibly contaminate food.

It is not known how many strains of Staphylococcus aureus produce this enterotoxin, and identification of these toxic strains depends on either injection or feeding of suspected material to susceptible experimental animals. As little as 2 to 10 ml of the enterotoxin can give rise to acute gastroenteritis in humans. It has been suggested that by

heating at 60°C for a minimum of 30 minutes the toxin will be inactivated, while others state that the enterotoxin is not destroyed by 100°C for 30 minutes (Wilson and Miles, 1961; Bryan, 1969; Bergdoll, 1969; de Figueiredo, 1969 and Angelotti, 1969). Since this organism was isolated from the muscles and tests are not available to rapidly identify enterotoxin-producing strains, it is advisable to cook the meat at least 30 minutes or preferably longer at a temperature no less than 100°C. It should be mentioned also that, even though the meat may look well cooked on the surface, the interior must also reach the desired temperature. Thus by reducing the thickness of meat, a higher temperature can be attained in a shorter time period. This is of importance also in the event that electricity is not available and cooking as we now know it is not possible; cooking on an open fire and keeping the thickness of the meat to a minimum may reduce the incidence of this type of food poisoning.

No appreciable differences were noted in the bacteriological evaluation of muscle at time of slaughter or 5 days post-slaughter, indicating no greater susceptibility of the irradiated carcass to bacterial invasion (Tables Ia and Ib).

In conjunction with the bacterial evaluations, meat samples were taken also at the previously mentioned times for chemical evaluation. These determinations consisted of: 1) ether extract (lipid content), 2) moisture content, 3) crude protein, 4) soluble protein and 5) pH.

The basic biochemical process taking place in the muscle tissue is anaerobic glycolysis--the formation of lactic acid from glycogen in the muscles. At the time of death, the equilibrium between assimilation and dissimilation is interrupted, and the dissimilation processes continue.

During this period one of the events which occurs is the production of lactic acid, causing a decrease in muscle pH from the normal 7.4 to approximately 5.5. This change alters also the physical state of the muscle, especially the protein myosin (Briskey, Cassens and Marsh, 1970).

The results of analysis of variance of these chemical-property variables are shown in Table II. With respect to ether extract, moisture, crude protein or soluble protein no significant differences between irradiated and control samples were observed. Since there was no significant time treatment interaction (Table II) the data were pooled and a significant decrease ($P < .01$) in pH was observed at 5 days post-slaughter (Table III). Meat from the irradiated animals had a significantly higher ($P < .05$) pH at time of slaughter than meat from the controls. After death, muscle tissue becomes acidic due to the production of lactic acid. The lactic acid is produced at the cost of the glycogen content of the muscle and depends on the amount of glycogen in the muscle. At, or a little below pH 5.4, the enzymatic action becomes retarded since it is self-limiting and pH dependent. The differences in overall means can be seen in Table III. The irradiated animals had a consistently higher pH. Therefore, in theory, the amount of glycogen in the muscle could have been lower in relation to the control animals and/or the enzymatic activity could have been suppressed in some manner.

The taste panel evaluations were conducted as outlined by Byer and Abrams (1953) and the results are tabulated in Table IV. Flavor evaluations varied, and the judges' ability to choose the odd samples reached statistical significance in three of the six tests. Several hypotheses may be proposed as to why the flavor effect was not consistent:

TABLE II

ANALYSIS OF VARIANCE OF CHEMICAL PROPERTIES OF MEAT FROM SWINE EXPOSED TO 700 R AT 1 R/MIN

Source	d.f.	Means Square				
		Ether Extract	Moisture	Crude Protein	Soluble Protein	pH
Total	13	-	-	-	-	-
Irr. vs Control	1	0.0802	0.0005	0.1498	5.5601	0.0778[c]
Animal/Tmt.[a]	5	4.7473	2.2873	0.5591	87.0797	0.0097
Time (Days)	1	5.8761	0.2471	0.9154	7.2864	0.8331[d]
Time x Tmt.	1	0.1254	0.0745	0.0957	1.1521	0.0003
Animal x Time/Tmt.[b]	5	0.9393	0.6665	0.1902	134.0733	0.0093

[a]Used as error term for irradiated vs controls.

[b]Used as error term for time x tmt., time and animal/tmt.

[c]Significant at the .05 level of probability.

[d]Significant at the .01 level of probability.

TABLE III

OVERALL MEANS OF CHEMICAL PROPERTIES OF MEAT FROM SWINE EXPOSED TO 700 R AT 1 R/MIN

Chemical Property	Treatment Effect		Time Effect Post-Irradiation	
	Control	Irradiated	Day 0	Day 5
Ether Extract (%)	4.44	4.27	4.96	3.67
Moisture (%)	74.18	74.17	74.04	74.31
Crude Protein (%)	18.86	19.08	18.76	19.27
Soluble Protein (mM leucine/10 g)	44.62	46.02	44.90	46.34
pH	5.56 a	5.73	5.76 b	5.61

[a]Between means indicates their difference to be significant at the .05 level of probability.

[b]Between means indicates their difference to be significant at the .01 level of probability.

TABLE IV

TRIANGLE SENSORY PANEL EVALUATION OF MEAT FROM IRRADIATED[a] AND NONIRRADIATED SWINE

Pork Roast (Test No.)	No. of Judges	Level of Statistical Significance[b]	
		Flavor	Color
1	10	NS	.01
2	9	.05	.01
3	9	NS	.05
4	9	.05	.01
5	9	.01	.01
6	9	NS	.01

[a]Animals received 700 R at 1 R/min.

[b]Measurement of ability of panel members to identify the odd sample.

1) the degree of radiation sickness of the animal, 2) the sensitivity of the individual panel members and/or 3) variation from animal to animal. In all the studies conducted, the panel noted a statistically significant difference in the appearance (color) of the meat samples. The probable reason for this was vascular injection occurring in the muscle of the irradiated animals.

It should be noted that during the course of this evaluation the psychological effect of tasting meat from animals exposed to radiation was quite apparent. During the screening of taste panel candidates, a large number of individuals indicated that they would not participate because the animals had been irradiated.

B. High Dose Rate Animals (45 R/min)

The animals receiving the high dose rate (45 R/min) were bled 1 and 3 days pre-irradiation and 2, 4, 6 and 9 days post-irradiation for the detection of a bacteremia. The results of the bacterial evaluations of the blood are summarized in Table V. In contrast to the low dose rate and control animals which showed no bacteremia, all of the high dose rate irradiated animals developed a bacteremia at least once during the 10-day post-irradiation period. However, the bacteremia was not consistent with respect to individual animals. Only one animal yielded positive blood cultures more than once and this occurred on the sixth and ninth days. The bacteremia detected earliest was found 4 days post-irradiation.

The animals were not bled on a day-to-day basis, but considering genera of bacteria isolated, the bacteremia appeared to have peaked on the sixth day and decreased by the ninth day. Several hypotheses can

TABLE V

BACTERIA ISOLATED FROM BLOOD OF IRRADIATED ANIMALS RECEIVING 700 R AT 45 R/MIN[a]

Days Post Irradiation	Positive Cultures (No./Total)	Positive Cultures (%)	Summary of Bacteria Isolated
4	2/9	22	β-hemolytic Streptococcus Proteus sp.
6	3/9	33	Bacillus subtilis Staphylococcus aureus Staphylococcus albus Sarcina sp. Alcaligenes faecalis Escherichia coli Aerobacter aerogenes Proteus sp.
9	2/9	22	Staphylococcus aureus Escherichia coli Pseudomonas sp. β-hemolytic Streptococcus

[a]All control animals yielded negative blood cultures.

be proposed concerning the high incidence on the sixth day and the inconsistency of the bacteremia in the animals. The high incidences of various bacteria isolated from the blood could have been attributed to the animals' environment, i.e., the contact the animals had with their food, water or other animals, or changes in the intestinal flora with a change in electrolyte balance in the gastrointestinal tract (a loss of fluid and electrolytes). Dehydration taking place and possibly retarding bacterial migration into the blood could also explain the inconsistency of the bacteremia. Also, the fixed and free macrophages of the body are rather radioresistant and can continue to ingest material after irradiation. The fixed macrophages in the loose connective tissues or lining the sinuses of the liver, spleen, lymph glands and bone marrow may still have had the ability to render the blood sterile. This does not agree with Chrom (1935) who concluded that the fixed reticuloendothelial cells of the liver and spleen were injured by irradiation and their filtering action thus impaired.

During the course of the bacteremia, various bacterial organisms may also have developed focal infections in various tissues and/or organs and the body was unable to completely destroy them, thus causing positive bacterial isolates from the lymph nodes, liver and muscle. It should be noted also that three of the animals developed a hematuria 5 days post-irradiation.

Results of the bacterial evaluations of the carcasses, at slaughter and 5 days post-slaughter are tabulated in Tables VIa and VIb. Numerous bacteria isolated from the blood were found in the various tissues cultured, indicating that the previous hypothesis may be correct.

TABLE VI

BACTERIA ISOLATED FROM CONTROL AND IRRADIATED SWINE SLAUGHTERED 10 DAYS AFTER EXPOSURE TO 700 R AT 45 R/MIN

Tissue	Positive Cultures (No./Total)	Positive Cultures (%)	Summary of Bacteria Isolated
a. Irradiated Swine			
Lymph Nodes (mesenteric)	6/6	100	Staphylococcus aureus Staphylococcus albus β-hemolytic Streptococcus Non-hemolytic Streptococcus Escherichia coli Proteus sp. Aerobacter sp. Bacillus subtilis
Liver	3/6	50	β-hemolytic Streptococcus α-hemolytic Streptococcus Staphylococcus aureus Bacillus subtilis Escherichia coli
Muscle[a]	5/6	83	Staphylococcus aureus Staphylococcus albus β-hemolytic Streptococcus Non-hemolytic Streptococcus Escherichia coli Bacillus subtilis Proteus sp.
Muscle[b]	3/6	50	Staphylococcus aureus β-hemolytic Streptococcus Pseudomonas sp. Proteus sp. Bacillus subtilis

TABLE VI (continued)

Tissue	Positive Cultures (No./Total)	Positive Cultures (%)	Summary of Bacteria Isolated
b. Control Swine			
Lymph Nodes (mesenteric)	2/2	100	Non-hemolytic *Streptococcus* β-hemolytic *Streptococcus* *Proteus sp.* *Escherichia coli* *Bacillus subtilis*
Liver	2/2	100	α-hemolytic *Streptococcus* *Escherichia coli*
Muscle[a]	1/2	50	*Pseudomonas sp.*
Muscle[b]	0/2	0	-

[a]Sampled at time of euthanasia.

[b]Sampled 5 days post-slaughter.

Comparison of the mesenteric lymph nodes shows that several different genera were isolated from the irradiated group. Again, as in the low dose rate group (1 R/min), one of the organisms most consistently isolated was Staphylococcus aureus. This organism was isolated from over 50% of the organs cultured, as well as from the blood. Since the lymph nodes act as filters in the body and positive cultures from the control animals also were observed, it is postulated that some migration from the intestinal lumina occurs naturally and thus yields positive mesenteric lymph node cultures.

Comparison of liver culture results again shows a lower incidence but a greater genera of bacterial isolated in the irradiated group. Since the liver also acts as a filtering mechanism of the body and a bacteremia was observed, it appears that a number of these organisms were retained but not destroyed by the liver. Two modes of bacterial distribution may have occurred: 1) entry into the portal circulation without being detected (as hypothesized with respect to results from the low dose rate group) and subsequent migration into the blood and invasion of other parts of the body where it again localizes (pyemia); or 2) a massive infiltration into the systemic circulation and then localizing in various organs and/or tissues. The bacterial evaluation of muscle from the irradiated animals yielded a high percentage of positive cultures at time of slaughter and the percentage was slightly less at 5 days post-slaughter. Only one bacterial genera was isolated from muscle of one control while seven and five genera were isolated from muscle 1 and 5 days after slaughter of the irradiated swine.

Several statements concerning the use of this meat from irradiated animals for human consumption are in order. Since staphylococcal food

poisoning has been discussed previously, it will be omitted at this time. With this large genera of bacteria isolated, inference should be made to nonspecific bacterial food poisoning. Organisms belonging to the Proteus, Bacillus, Streptococcus or Bacterium groups have all been incriminated in food poisoning. It is not known if the organisms secrete a true enterotoxin, produce toxic breakdown products in the medium and/or alter the normal bacterial equilibrium.

Whatever mode or modes of action prevail, it is of prime importance to note that these organisms can cause food poisoning. This meat must be regarded as potentially infective in man and should not be eaten unless thoroughly cooked. Cooking, however, does not necessarily sterilize the meat. The penetration of heat into a large muscle area is slow and the interior of the meat (depending on size) may not reach and/or be kept at the optimum temperature long enough to destroy all the organisms. Also, the degree of heat necessary to destroy the organisms is dependent on the type of medium, i.e., ground or intact meat. If possible, the meat should be kept cold till cooking and if the meat is not consumed immediately after cooking it again should be cooled as quickly as possible. In the event that no electrical power is available for chilling and storage of this meat (as might be the case in a post-attack situation), it is recommended, from a bacterial standpoint, that this meat not be consumed.

It appears also from data in Tables VIa and VIb that the carcasses from irradiated animals are more compatible for bacterial growth during storage. Possible reasons for this also have been previously discussed for the low dose rate group.

The results of analysis of variance results of the chemical variable determined from the meat of swine exposed to 45 R/min are tabulated

in Table VII. The irradiated and control animals were significantly different with respect to ether extract and moisture content at time of slaughter ($P < .05$) as shown in Tables VII and VIII.

The fat content of animal tissues varies widely, not only with respect to type of tissue but also with respect to sex, age and nutrition. Lipids or fat can be divided into two major groupings: 1) simple triglycerides or neutral fat, which is the most predominant type deposited under the skin and in body cavities and 2) compound lipids such as phospholipids found in certain vital organs such as the heart and liver.

The visible outer fat layer of the meat was removed manually prior to grinding in order to better evaluate the intramuscular lipid content, for it is believed that lipid stored in or near the muscle cells could serve as energy sources for the muscle. If this hypothesis is correct, it appears that some biochemical pathways were retarded or altered, inhibiting the muscle in its mobilization of energy from this source. Comparison of the overall means in Table VIII indicates a greater percentage of ether extract recovered from the irradiated group which could be considered as evidence in support of the above hypothesis. Comparison of the time effect (Table VIII) shows that at 5 days post-slaughter a higher level of ether extract was present in the muscle than at the time of slaughter. The probable explanation for this increase is the decrease in moisture content which would affect the lipid content of muscle. It should be mentioned also that anhydrous diethyl ether was used in this determination, and the extracted fraction not only contained fats and oils but also phospholipids, sterols and fat soluble vitamins which could possibly alter the results.

TABLE VII

ANALYSIS OF VARIANCE OF CHEMICAL PROPERTIES OF MEAT FROM SWINE EXPOSED TO 700 R AT 45 R/MIN

Source	d.f.	Means Square				
		Ether Extract	Moisture	Crude Protein	Soluble Protein	pH
Total	15	-	-	-	-	-
Irr. vs Control	1	16.9456[c]	6.0990[c]	0.0690	25.2300	0.2852[c]
Animal/Tmt.[a]	6	1.6492	0.7661	0.9130	20.2400	0.0395
Time (Days)	1	14.3262[c]	10.7092[c]	0.0841	103.0225[d]	0.0110
Time x Tmt.	1	2.4031	2.9850	0.0408	99.1875	0.0520
Animal x Time/Tmt.[b]	6	1.3522	0.8934	0.1546	17.1633	0.0203

[a]Used as error term for irradiated vs controls.

[b]Used as error term for time x tmt., time and animal/tmt.

[c]Significant at the .05 level of probability.

[d]Significant at the .01 level of probability.

TABLE VIII

OVERALL MEANS OF CHEMICAL PROPERTIES OF MEAT FROM SWINE EXPOSED TO 700 R AT 45 R/MIN

Chemical Property	Treatment Effect			Time Effect Post-Irradiation		
	Control		Irradiated	Day 0		Day 5
Ether Extract (%)	4.24	a	6.62	5.08	a	6.97
Moisture (%)	74.04	a	72.62	73.79	a	72.16
Crude Protein (%)	18.78		18.93	18.96		18.82
Soluble Protein (mM leucine/10 g)	42.02		44.92	46.74	b	41.66
pH	5.48	a	5.79	5.74		5.69

[a]Between means indicates their difference to be significant at the .05 level of probability.

[b]Between means indicates their difference to be significant at the .01 level of probability.

The moisture content of muscle performs many important functions in the body and is a major and integral part of all living protoplasm. The irradiated animals had a significantly lower moisture percentage than the control animals. Cells contain two forms of water: 1) free water which contains the dissolved solutes and serves as a dispersion medium in the protoplasm and 2) bound water in which the water is attached (bound) to colloidal particles. Cellular activities are regulated to an extent by the bound/free water equilibrium and under certain conditions of stress, it is possible to shift this equilibrium in one direction or the other (Mertz, 1959). It is hypothesized that the above occurred, since the irradiated animals were under stress, which would cause a decrease in moisture content.

No significant differences were observed with respect to percentage of crude protein, but a highly significant ($P < .01$) difference between the two times was observed in millimoles of soluble protein (see Table VIII). The colorimetric ninhydrin method used for this determination is not specific for just the NH_2 groups of amino acids, but also reacts with the NH_2 group in peptides, proteins and other classes of substances possessing free amino groups. It is possible that in some way not yet understood, a reduction of so-called free NH_2 reacting sites occurred during the storage period and thus yielded a lower soluble protein value.

As was observed in the low dose rate group, the irradiated animals had a higher pH value at time of slaughter than the control animals. It is again hypothesized that the glycogen content of the muscle in the irradiated animals was lower than in the control animals, or that some enzymatic pathway was retarded. Also, both of the above could have

occurred simultaneously. However, no pH change with time was observed.

Results of the taste panel evaluation are tabulated in Table IX. Flavor and color differences were, on the whole, apparently less pronounced than in the low dose rate group. These results seemed unusual in that it was assumed a priori that a more pronounced effect would be observed. However, two factors must be considered: 1) panel members may have had a preconceived notion that the meat to be evaluated was from irradiated animals, which could have affected their judgement, and 2) the flavor effect may not have occurred as yet, due to the high dose rate imposed. This does not explain in full the color results, for it appeared that the hemorrhages which occurred in the high dose rate group were much more apparent. The possibility that the cooking and/or handling of the meat was not exactly the same for the low and high dose rate group also must be considered.

C. Rat Experiment

Since a bacteremia was not observed in swine of the low dose rate group (1 R/min), a pilot study was conducted to evaluate the previously mentioned procedure with rats. The literature indicated that bacteremias occurred in laboratory animals exposed to whole-body irradiation.

The results concerning bacteria isolated from the blood of rats receiving variable doses at 50 R/min are summarized in Table X. Blood samples were taken via cardiac puncture when the rats showed visible signs of radiation sickness or when in the moribund state. It appears that the procedure used for the collection of sterile blood was quite satisfactory for large and small animals. The results obtained in this bacteriological study indicate that as the total dose increased so did

TABLE IX

TRIANGLE SENSORY PANEL EVALUATION OF MEAT FROM IRRADIATED[a] AND NONIRRADIATED SWINE

Pork Roast (Test No.)	No. of Judges	Level of Statistical Significance[b]	
		Flavor	Color
1	8	.05	.05
2	8	NS	NS
3	6	.01	.01
4	6	NS	.05
5	8	NS	.01
6	8	NS	NS

[a]Animals received 700 R at 45 R/min.

[b]Measurement of ability of panel members to identify the odd sample.

TABLE X

BACTERIA ISOLATED FROM BLOOD OF RATS RECEIVING VARIABLE DOSES AT 50 R/MIN[a]

Dose (R)	Positive Cultures (No./Total)	Positive Cultures (%)	Summary of Bacteria Isolated
1,000	9/11	82	Escherichia coli Pseudomonas sp. Proteus sp. Bacillus subtilis β-hemolytic Streptococcus
900	10/13	75	Escherichia coli Aerobacter aerogenes Bacillus subtilis β-hemolytic Streptococcus λ-hemolytic Streptococcus
800	9/15	60	Escherichia coli Aerobacter aerogenes Proteus sp. β-hemolytic Streptococcus Staphylococcus aureus

[a]All control rats had negative blood cultures.

the incidence of bacteremia. Similar results were reported by Boone, Woodward and Harris (1956), Gonshery, Marston and Smith (1953) and Osborne et al. (1952). The percentage of positive cultures might have been higher in the 900- and 1,000-R groups if some of the animals had not succumbed prior to or during the bleeding procedure.

D. General Discussion

In the event of a nuclear disaster rapid recovery is of prime importance for survival and national stability. Recovery requires safe and adequate food supplies for the survivors, for without this, recovery is only limited and time dependent. History has shown over and over that food directly influences the work potential and morale of the people. The use of animals exposed to ionizing radiation could greatly help supply our need for food.

The pig (Sus scrofa domestica) was used as the experimental animal for several reasons: 1) most swine have some form of housing, due to confinement feeding practices, 2) only a fraction of the swine population would be out on pasture and 3) since the animals are housed, the major radiation effect would be exposure to gamma rays. Thus, the use of this animal would appear feasible in view of the criteria established for evaluation of a post-attack situation. However, with respect to the overall picture, it should be remembered that pigs are not our only meat commodity, for cattle are just as important or moreso, but are more vulnerable due to the fact that most of these animals are not sheltered (as compared to pigs) but are out on pastures. Here they are subjected not only to whole-body gamma radiation effects but also to the ingested and surface-retained fallout of a nuclear attack. However,

it was not within the scope of this study to evaluate the role of cattle in a post-attack situation. The reader should be aware, however, that cattle, as well as swine, must play an important role in supplying food for human utilization.

When data has been accumulated on a few species, many will try to extrapolate it to other species. In this situation it would not be in the best interest (author's own opinion) due to a large number of variables which would be encountered, such as differences in susceptibility and natural immunity to various bacterial organisms, environment, digestive tracts (monogastric as compared to ruminant) and many other possible differences.

What does all this mean? It means that one of this country's most valuable and important assets, i.e., farm animals, are quite vulnerable during an attack and in the post-attack environment. Hardships imposed on the people will be at a maximum and food supplies scarce (in some geographical areas). As was mentioned in the introduction, only when food supplies become scarce does man realize his great dependence on agriculture and its products. In order to minimize our post-attack problems, more research and information is needed to better evaluate the role farm animals will and must play. Thus, new guidelines should be formulated with the purpose of informing the population on the safety of this meat for human consumption.

CHAPTER V

SUMMARY AND CONCLUSIONS

Animals showing visible signs of radiation sickness could be an important food source in the event of a nuclear disaster if they could safely be used for human consumption. The initial objective of this study was to determine if irradiated swine develop a bacteremia and whether there was further bacterial invasion of the organs and/or muscles. In conjunction with the bacterial evaluations, chemical evaluations of the meat were conducted to determine if any gross chemical changes occurred which could possibly indicate that meat from irradiated animals is unfit for consumption. Sensory panel evaluations were conducted to determine if any flavor and/or color changes occurred which might be related to the chemical evaluations. Pigs weighing approximately 225-250 lb were subjected to a total (air) dose of 700 R, at 1 R and 45 R/min, of gamma radiation from a ^{60}Co source. Bacteriological studies were conducted on the blood (pre- and post-irradiation) as well as muscle, liver and lymph nodes (mesenteric) 10 days post-irradiation when the animals were slaughtered.

In the low dose rate group (1 R/min) no evidence of a bacteria was observed, indicating that the reticuloendothelial system was not impaired to the extent that it was incapable of maintaining sterility of the blood if bacteria entered the circulatory system. Comparison of the carcasses at time of slaughter and 5 days post-slaughter indicated no greater susceptibility of the irradiated carcass to bacterial invasion. The organism Staphylococcus aureus was recovered from the

meat and several strains of these bacteria are capable of producing an enterotoxin which is toxic to humans and, upon ingestion by them, causes acute gastroenteritis. Since no tests are available to rapidly identify the enterotoxin-producing strains, it is advised to cook the meat for a minimum of 30 minutes, preferably longer, at a temperature of no less than 100°C, thus increasing the likelihood of inactivation of the toxin.

The results of analysis of variance of various chemical properties indicated no significant differences in ether extract, moisture content, crude protein or soluble protein. A significant difference was observed in the pH of the irradiated and control animals at the time of slaughter. Irradiated animals had a consistently higher pH than the respective control animals. The pH significantly decreased with storage. The taste panel evaluations conducted indicate no drastic flavor changes. The flavor effect varied, being statistically significant in some comparisons. In all studies conducted, the panel noted a statistical difference in the color of the meat samples, but not to such a degree that it was considered undesirable.

Thus, on the basis of these data, it is believed that the low dose rate irradiated animals could be utilized for human consumption. It should be stated, however, that if the carcasses had a higher incidence of bacterial isolates (i.e., food poisoning bacteria) the utilization of the meat would be questionable.

All animals exposed to the high dose rate (45 R/min) developed a bacteremia at least once during the 10-day post-irradiation period. However, the bacteremia was inconsistent from animal to animal. Results of bacterial evaluations of the carcasses, at slaughter and 5 days

post-slaughter, yielded a higher percentage of bacteria in muscle from irradiated animals than in that from control carcasses. At slaughter liver and lymph nodes from the irradiated animals also yielded a greater number of genera of bacteria.

With this large number of genera of bacteria isolated from the muscle, consideration should be given to nonspecific bacterial food poisoning as well as to staphylococcal food poisoning. Such meat should be regarded as potentially infective for man and should not be consumed without thorough cooking (minimum time, 30 minutes at 100°C). If this is not possible, it is recommended, from a bacterial standpoint, that this meat not be consumed. With the 5-day storage of the carcass, a slight decrease in number of kinds (genera) of bacteria was noted. Possible spoilage, however, may be enhanced and nonspecific food poisoning bacteria may still be a threat. Thus, the previously stated recommendation for proper cooking should still be followed.

The results of the chemical determinations revealed significant differences with respect to ether extract (lipid content) and moisture content. A highly significant difference between times was observed with respect to soluble protein. A similar pH response was observed in these animals at time of slaughter as in the low dose rate group, but not after the storage phase. The sensory panel differences were, on the whole, less consistent for flavor and color than in the low dose rate group. These results seemed unusual. On a priori grounds a more pronounced effect had been expected.

The rat pilot study yielded two important results: 1) demonstration that the procedure used for the collection of sterile blood is quite

satisfactory and 2) the development of a bacteremia, which has been reported by previous researchers.

In conclusion, the animals exposed to the low dose rate could be utilized for human consumption in the light of the present data. At present, it appears unsafe to utilize meat from animals exposed to the high dose rate. It should be emphasized that our knowledge of this subject is still rather limited. More research is needed to fill critical gaps in our understanding and permit utilization of these animals (swine, cattle, sheep, poultry) to their maximum potential in a post-attack situation. Guidelines for the optimum time to slaughter, store and utilize this meat are needed, with one ultimate objective in mind: informing the population that these animals are or are not safe for human consumption and under what specific circumstances.

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APPENDIX

TABLE XI

INDIVIDUAL DATA OF CHEMICAL PROPERTIES OF MEAT FROM SWINE EXPOSED TO 700 R AT 1 R/MIN

Animal No.	Ether Extract	Moisture	Crude Protein	Soluble Protein	pH
At Slaughter:					
55T	4.45	73.01	19.52	56.6	5.85
57T	6.52	73.61	18.00	35.3	5.90
58T	4.85	75.41	18.08	48.6	5.85
59T (Control)	6.42	73.80	18.14	25.6	5.64
67T	6.49	72.01	19.04	37.3	5.82
68T (Control)	4.05	74.53	19.32	61.3	5.66
69T	1.98	75.91	19.24	49.6	5.60
5 Days Post-Slaughter:					
55T	1.85	74.43	19.76	46.3	5.78
57T	5.88	73.25	18.90	55.6	5.51
58T	4.57	74.49	18.84	42.0	5.69
59T (Control)	3.58	74.56	19.30	47.6	5.48
67T	4.05	73.81	19.66	40.6	5.68
68T (Control)	3.70	73.84	18.66	44.0	5.48
69T	2.06	75.76	19.80	48.3	5.62

TABLE XII

INDIVIDUAL DATA OF CHEMICAL PROPERTIES OF MEAT FROM SWINE EXPOSED TO 700 R AT 45 R/MIN

Animal No.	Ether Extract	Moisture	Crude Protein	Soluble Protein	pH
At Slaughter:					
55K	4.48	73.56	20.06	56.6	6.02
56K	5.60	73.66	19.42	55.3	5.58
57K (Control)	3.83	74.88	18.12	37.3	5.61
58K	4.47	74.81	18.30	41.9	5.74
65K	5.90	72.99	19.26	49.5	5.70
66K	5.67	73.94	18.21	43.8	5.89
67K (Control)	4.10	73.35	19.40	43.2	5.61
68K	6.56	73.16	18.92	46.3	5.79
5 Days Post-Slaughter:					
55K	6.62	72.05	19.60	41.6	6.16
56K	6.16	72.96	19.28	39.3	5.79
57K (Control)	4.01	74.77	18.47	42.3	5.44
58K	9.73	70.05	18.23	40.6	5.76
65K	6.46	71.96	19.51	41.3	5.78
66K	7.84	71.61	18.61	38.6	5.49
67K (Control)	5.02	73.18	19.11	45.3	5.28
68K	9.91	70.68	17.72	44.3	5.82

VITA

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After completion of high school in the Philadelphia area, he entered Delaware Valley College, Doylestown, Pennsylvania and received his B.S. in Animal Husbandry. Graduating in 1966, he then entered the University of Tennessee Graduate School and received his M.S. in Animal Science in 1968 and his Ph.D. in Animal Science in 1972.