

NAV1.950131.017



Edited by

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Research Institute

1942-1962

III. RADIATION BIOLOGY

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Ionizing radiation and its effect upon men and materials has developed into a major military problem. Radiation became a casualty producing agent with the introduction of nuclear weapons. Radiation is produced at the time of the explosion and continues with decreasing intensity from the residual radioactive materials and from neutron induced radioactivity. Rainout and fallout from atmospherically borne radioactive debris may contaminate huge land masses with psychologically annoying or lethal levels of ionizing radiation to human beings. Similarly ships and aircraft may become annoyingly or dangerously contaminated by operation in and through contaminated atmospheres or waters. For the Navy, radiation problems are not limited to the actual use of nuclear weapons in warfare. The development of ship propulsion by nuclear reactor generated power is spreading throughout the fleet. From nuclear weapons and the use of nuclear reactors for power, naval personnel may be exposed to small doses of gamma rays and neutrons at all energies over long periods of time. Reactor accidents although highly unlikely may expose naval personnel to high doses over a short period. A problem unique to the Navy is the concentration of certain fission products in marine life growing on the ships' bottoms. In peacetime maneuvers and traifing there is a hazard from the components of nuclear reactors, radiation from spent fuel rods, leaks in nuclear weapons systems (tritium gas), accidental nonnuclear explosions of nuclear weapons systems (contamination of wounds, inhalation or ingestion of plutonium and uranium). These are just a few of the potential radiation problems with which the Navy of today is confronted. Exposure to radiation may result in acute injury or may produce injury that remains hidden in the information system of proliferating cells that is

expressed years later as leukemia, diminution in longevity, cataracts, etc.

The understanding of ionizing radiation in all its forms and knowledge of its effects on living things commenced less than three score and ten years ago. In 1895 Roentgen discovered X-rays. In 1900 Kassarbian commenced the description of radiation dermatitis of his hands that would later result in radiation cancer followed by amputations and ultimately his death in 1909. In 1905 Einstein formulated the theory of relativity which laid the theoretical background for the ultimate development of nuclear energy. In the period 1895 through 1940 there was intensive study of radiation effects on living tissue. These were pursued vigorously, particularly in France and Germany initially, and later England, the United States, and elsewhere. Of particular note are the pathologic and hematologic studies of Heinecke, Laccasagne, Fabricius-Møller (Denmark), and many others which form the foundation upon which qualitative pathologic and hematologic effects of radiation are still based. In this period, however, studies were primarily focused upon local irradiation and were generated from the desire to improve radiation therapy of cancer in addition to obtaining a basic understanding of the mechanism of the effects of ionizing radiation upon tissue. In 1932 the introduction of the roentgen unit as an adequate measure of the radiation delivered in air brought radiation biology to the level of a quantitative science. The neutron was discovered by Chadwick in 1932. Nuclear fission of uranium was observed by Hahn and associates in 1939. In this year Einstein communicated to President Roosevelt the ideas of nuclear physicists upon the military possibilities of atomic energy and bombs. Government support of fundamental studies on nuclear fission was

third priority to detonation at a depth many thousands of feet beneath the surface. The specific objective of the tests would be to ascertain the strategic and tactical significance of the atomic bomb as affecting the future composition and employment of armed forces and determine what changes would be required in naval design and construction. Since ionizing radiation had become clearly a new type of casualty producing agent, biological studies were to be an integral part. The Naval Medical Research Section of Joint Task Force One was organized under Rear Adm. T. A. Solberg, USN, Director of Ship Material, by, Capt. R. H. Draeger, MC, USN, of the Naval Medical Research Institute, who was appointed as Commanding Officer and Capt. Shields Warren, MC, USNR, who was appointed as Executive Officer of the Naval Medical Research Unit. As a result of his known distinction in radiation pathology and participation in these tests, Warren later became the first Director of Division of Biology and Medicine of the U.S. Atomic Energy Commission. The U.S.S. *Burlason*, APA 67, was designated as animal and laboratory ship and ordered to the U.S. Naval Shipyard, San Francisco, for remodeling into a sophisticated laboratory and animal quarters afloat, after designs by Capt. R. H. Draeger, MC, USN and Lt. S. Scal, MC, USNR.

The biomedical program was designed to test the effectiveness of radiation against living animals and to study thermal burns and blast effects. Aboard the ship were 200 swine, 204 goats, 60 guinea pigs, 5,000 rats, and 200 mice to be used in the studies. Major accomplishments of the radiobiological program were: (a) The determination of the inability to predict satisfactorily the dose of radiation within ships thus demonstrating the absolute necessity for development of satisfactory dosimetric techniques. (b) That radiation could be a lethal agent under conditions where ships are not destroyed and individuals beneath decks are protected from thermal and traumatic injuries. (c) Several new dosimeter devices and methods were tested and shown to be of limited effectiveness. (d) Following test BAKER, the underwater shot, the highly lethal nature of radiation from the base surge and rain-out over a wide area was demonstrated in ships, with animals beneath decks, in which the ships suffered minor physical damage but in which the animals died within 3 to 4 days following the exposure from pure radiation injury. (e) The flora and fauna which grow upon the bottoms of naval vessels were shown to concentrate significant radioactivity from the contaminated sea water. (f) The large animal studies at Operation CROSSROADS clearly demonstrated the feasibility of

dividing radiation injury into various categories depending upon the rapidity of the development of leukopenia and the tempo with which the signs and symptoms appear. (g) Furthermore, it appeared, although there were inadequate control studies, that antibiotics and fresh blood transfusions were beneficial. (h) The predominance of gastrointestinal symptomatology and injury in the very high dose groups was suggested. (i) In the lethal dose range where there was a spontaneous possibility of survival, the predominance of overwhelming infection was conclusive. The pathology and lethal dose studies clearly showed that an underwater atomic bomb explosion produces much more lethal ionizing radiation over a larger area due to the base surge and the radioactivity that comes down in the rainout. (j) Pathology studies gave confirmation of the clinical and hematological studies during life. The histopathological studies of Lt. Comdr. J. L. Tullis, MC, USN, on the Bikini animals pointed out for the first time an important exception to the law of Bergonie and Tribondeau, namely, that the stem cells of the lymphoid organs, the bone marrow, the testes, and the ovaries are more resistant than the more mature cells. This important basic observation in radiation biology was made as a result of applied studies in the field.

Partial or complete failure to attain certain objectives clearly define certain problem areas: (a) The quantitative relationship between the dose of radiation and ultimate mortality in different species of animals was not determined because of failure of radiation dosimetry and/or the difficulty of computing the dose beneath such a complicated shielding system as exists in naval vessels thus demonstrating a great need for development of refined dosimetry of the initial bomb radiation and residual radiation fields. (b) The complicated shielding configurations from ships' compartments, bulkheads, and machinery which "shadow shielded" animals demonstrated the necessity for performing inhomogenous irradiation of animals. (c) The necessity for dose rate and lethal dose studies combined with histopathologic and hematologic studies was clearly demonstrated after the BAKER contaminating test. (d) The presence of combined thermal, mechanical, and radiation injuries was shown clearly thus indicating a new field for study in the laboratory.

The serious health problems of surface ship radioactive contamination, and entrance of fission products into the life cycle of marine flora and fauna growing on ships' bottoms were problems that could not be handled at NMRI and necessitated a new laboratory embracing a cross-disciplinary attack of physics, chem-

istry, engineering, and biomedicine. Thus the U.S. Naval Radiological Defense Laboratory was born.

The positive accomplishments and the failures to attain some objectives clearly aided in the design of continuing studies at NMRI in preparation for later field testing if further nuclear bombs were to be exploded. Upon the return of the Naval Medical Research team to NMRI, the Atomic Medicine Division under Capt. R. H. Draeger, MC, USN and Comdr. R. H. Lee, MSC, USN, was formed in order to plan for further field tests. Intramurally systematic pathological and hematological studies on swine, goats, dogs, and small mammals in order to dissect systematically the pattern of radiation injury as a function of dose, percent mortality, varying depth dose patterns, and varying dose rates were commenced. In conjunction with these investigations, comprehensive depth dose studies were instituted by the Radiation Technology Division under Comdr. W. H. Chambers, Jr. MSC, USN.

The internal collaborative studies between divisions at NMRI rapidly began to bear fruit. As a result of collaboration between the Pathology and Radiation Technology Divisions, the vital role of differing depth dose patterns upon the mortality of radiation was demonstrated clearly. For the same amount of radiation delivered in air a more homogenous distribution within tissue is significantly more lethal. These studies clearly pointed out the necessity of eventually determining the depth dose pattern of the initial neutron and gamma radiation from nuclear bombs and the depth dose pattern from radiation that might develop in any military situation in order to assess the military hazard. Thus one was confronted with two primary and equally important problems. First, it was necessary to work out techniques for delivering reproducible depth dose patterns in animals from whole body radiation in order to study hematological and pathological effects and to evaluate potential therapeutic or prophylactic agents. Second, it was necessary to work out the techniques for practical determination of depth dose patterns for the laboratory which could be used in the field while testing future atomic bombs.

For the dosimetry, the Radiation Technology Division developed small Sievert type ionization dosimeters that could be placed at successive depths in tissue equivalent phantoms for measuring the tissue dose. In addition, a sophisticated array of film and phosphor dosimeters were also developed for use in parallel with the small ionization chambers. With these techniques, precise measurements of the distribution of the dose

within tissue equivalent phantoms were made for the 1-Mev X-ray machine at the Naval Gun Factory, the 2-Mev X-ray machine at the Naval Ordnance Plant, and the 250-kvp therapy unit at NMRI. In addition they were utilized to measure the air dose and scatter from the exposure equipment under different conditions of scattering material. The role of scattered radiation and the careful design of exposure equipment to assure reproducibility and to include scattered radiation in the biologic effects were successfully exploited by F. Ellinger. It was shown early that the most practical means of having a reproducible depth dose pattern in animals of varying sizes was to deliver half the dose to each side of the animal. This technique was thoroughly evaluated from a biological standpoint and shown to be acceptable for controlled studies of radiation mortality and therapy of radiation injury and has now become a standard technique in radiation biology laboratories.

The radiation from a nuclear bomb is directional and it was necessary to try to simulate bomb radiation. In order to accomplish this it was first necessary to await field tests in order to determine the depth dose pattern of the initial radiation from a nuclear bomb and also to see if depth dose pattern varied with distance from the explosion point. An opportunity to initiate these studies would not appear until 1951 during Operation GREENHOUSE.

Between 1946 and 1949 a broad biological program on the hematological and pathological effects of radiation and the modification of radiation injury by chemical means was launched jointly by the Hematology Division (E. P. Cronkite) and Pathology Division (J. L. Tullis). For chemical prophylaxis against radiation a program was developed for study of *sulfhydryl* compounds which was based upon the works of E. S. G. Barron of the University of Chicago (consultant to NMRI) who had demonstrated that the addition of glutathione (GSH) to enzymes would afford considerable protection against the inactivation of these enzymes by radiation in vitro. However, in order to evaluate the effect of glutathione (or other substances) it was first necessary to establish precise methods of bioassay of radiation effects. This involved not only precise techniques for the irradiation of the animals but also a very careful randomization and statistical selection to avoid introducing bias into the experiments, and careful maintenance to prevent introduction of infection into the irradiated colony after exposure. Utilizing the radial beam of the 2-Mev X-ray machines at the White Oaks Naval Ordnance Laboratory it was possible to expose animals at dose rates

of 32 r per minute and simultaneously expose up to 512 animals so that an entire LD₅₀ curve could be determined simultaneously for the treated and control animals. It was demonstrated that glutathione when administered prior to irradiation would significantly protect, almost doubling the LD₅₀ dose of irradiation. However, the amounts of glutathione that produced this degree of protection verged on being lethal themselves. Independently and shortly before demonstration of protection by glutathione at NMRI, the Argonne National Laboratory demonstrated protection by intravenous cysteine. These were the first two clear-cut demonstrations of the capability of protecting against radiation by the administration of chemical substances prior to irradiation. Systematic studies were performed on the nature of the sulfhydryl protection. It was demonstrated that the protection was obtained only while a significantly increased concentration of sulfhydryl compounds existed within radio-sensitive tissues essential to life. It was further shown that only those tissues that concentrate the sulfhydryl compounds are protected. For example, the testicle does not increase the concentration of glutathione following its administration and is not protected. The preceding was based upon extensive studies of tissue distribution and plasma clearance in diverse species and then selected studies upon the rate of development of atrophy of tissues following irradiation.

When a clinically interesting compound was reported in the literature as having a protective effect against radiation, it was checked with the techniques available at NMRI for assay in irradiated mice. It was successively demonstrated that none of the rutin-flavonoid group of compounds were of benefit as claimed by others. It was further demonstrated that vitamin B₁₂, folic acid pyridoxine, and other substances reputedly of benefit were in reality, when adequately tested in a statistically sound system, not protective.

Attempts were made to develop oral sulfhydryl compounds in conjunction with the Schwartz Chemical Co. but this was unsuccessful. The sulfhydryl protection which initially appeared promising and possibly useful in protecting military personnel against radiation was given up because of the high toxicity and the inability to maintain a prolonged protective level of sulfhydryl compounds in the tissues of irradiated animals. It is of interest that the entire sulfhydryl protection program was reopened by the U.S. Army Medical Department in an extensive program aimed at obtaining protective compounds. The pathological picture of sulfhydryl protection was performed in con-

nection with G. Brecher at the National Institutes of Health (NIH). This study demonstrated the anatomical site and the cells from which hematological regeneration commences and generated a long and fruitful series of collaborative studies between Hematology Division, NMRI, and Pathology Division, National Institute of Arthritis and Metabolic Diseases, NIH.

Also in this period the Pathology Division systematically studied the pathology of radiation injury particularly of the gastrointestinal tract as a function of the dose of radiation. Later studies by Capt. R. B. Williams, MC, USN, 1955-60, on the quantitative effects of radiation on cell proliferation in the gastrointestinal tract have become classical basic studies on the effects of radiation on mitosis, regeneration, and DNA synthesis in the bowel.

A clinical analysis of the reports of the Joint Commission and of the Atomic Bomb Casualty Commission were undertaken and from these and studies on laboratory animals the now useful clinical subdivision of the radiation syndromes were developed and later were incorporated into the USAEC's test on "Effects of Nuclear Weapons." It was shown for practical purposes that one can divide radiation injury of man into three categories: (i) survival improbable, (ii) survival possible, and (iii) survival probable. Simple clinical observations determine in which category a patient probably belongs thus setting a basis for triage in the event of mass radiation casualties. It was further shown simultaneously at NMRI and elsewhere that radiation syndromes in animals vary with the dose of radiation received and the time after exposure. After very high doses of radiation of the order of many thousands of roentgen units either to the head or to the whole body, a typical syndrome develops characterized by signs and symptoms associated with the central nervous system and thus was called the CNS syndrome. This appears either during or shortly after exposure and is uniformly fatal. After doses of 800-2000 r, a symptomatology develops that is characterized by gastrointestinal symptoms which include a stable survival time in mice of 3 to 4 days and has in general a 100 percent mortality in all laboratory animals. In 1953 and 1954, it was shown by Conrad et al. that extensive administration of plasma and fluids to dogs with otherwise fatal gastrointestinal syndrome, would prolong life by preventing death from shock and thus allow sufficient time for spontaneous regeneration of the bowel from the histological standpoint. This was the first demonstration of successful therapy of the gastrointestinal injury. The pathologic studies on

bowel regeneration were another extension of the collaborative work commenced earlier with NIH.

After doses of radiation in the lethal dose range it was clearly shown that the gastrointestinal symptomatology is relatively fleeting and that the cause of death is related to the aplasia of the bone marrow, producing successively, increased susceptibility to infection that may have a fatal outcome within 1 to 2 weeks and later an increased susceptibility to spontaneous bleeding. Thus in the lethal dose range the first cause of death may be infection and later exsanguinating hemorrhage or fatal hemorrhage into a vital organ. This classification of the radiation syndrome produced by whole body irradiation is in general use today.

The Pathology Division evaluated the effect of radiation upon phagocytosis by the reticuloendothelial system utilizing the clearance rate of radioactive colloidal gold from the blood stream. At no time following total body X-radiation was the rate of removal of radioactive gold from the circulation found to be significantly impaired and it was concluded that total body ionizing radiation injury in the lethal range does not influence significantly the capability of the RES system to phagocytize foreign material within the blood stream. This was the initial study on the effect of radiation upon the capability of the reticuloendothelial system to phagocytize. Subsequently, numerous studies in many laboratories throughout the world have essentially confirmed this original work.

The Dental Division under Capt. James English, DC, USN, extensively studied the effects of ionizing radiation upon developing teeth in rats and in swine. In addition, studies upon the composition of saliva were also made. These studies have become classics in the field of the effects of radiation on oral tissues.

Among the oral manifestations of total body irradiation as seen in Nagasaki and Hiroshima patients was the presence of acute fulminating necrotizing gingivitis plus ulceration of the buccal mucosa. It was apparent that more basic information was required. Collaborative studies with the Hematology Division, NMRI, showed that in total body X-ray irradiated dogs, ulcerative gingivitis developed during the early stage of hemopoietic depression, reaching the fulminating stage as the animals became moribund.

The Bikini test trials presented an opportunity to observe the effects of high energy gamma irradiation. These studies showed that the ameloblast was especially susceptible to injury, having a pronounced effect on the developing tooth. Hemorrhage within the follicular sac was a common observation.

The effects of bilaterally applied X-rays to the head

and neck of dogs, in doses ranging from 1000 to 1750 roentgens, yielded further valuable information. Salivary gland parenchyma showed evidence of severe injury, followed by bizarre changes in glandular cell architecture. Cell damage proved to be irreversible at higher dose levels. Dosimetry measurements indicated summation at the midline.

The metabolism of exteriorized salivary glands in the rat was affected by X-ray irradiation, these changes being especially noticeable in various enzyme systems.

Field studies at Frenchman's Flats revealed that many dental materials became dangerously radioactive after capture of thermal neutrons when released by nuclear explosions. Current studies are now in progress regarding the effect of thermal and fast neutrons on oral tissues and dental restorations.

A series of studies was performed on irradiated animals in order to determine whether radiation was different from any other type of stress. It was clearly shown that radiation did not differ significantly from other types of stresses, that adrenalectomy sensitizes animals to irradiation, and that there is a significant increase in excretion of 17-ketosteroids in the urine.

The Pathology Division (Brown, Hardenbergh, and Tullis) systematically studied the influence of irradiation upon the biochemical, cellular, and bacteriological content of thoracic duct lymph and blood in normal dogs and in dogs exposed to 500 r total body X-radiation. The white blood cell concentration of lymph dropped precipitously and attained minimum values within 4 hours after radiation and remained at this low level throughout the period of observation extending as long as 4 days after radiation. The cultures from blood and lymph remained sterile indicating that phagocytosis was not impaired since the animals did develop known infections.

In 1949 Operation GREENHOUSE was established as a joint Army-Navy-Air Force operation for the study of nuclear bomb effects. This turned out to be the operation in which the first thermonuclear bomb was exploded. Biomedical studies were a major part of the operation. Since the operation was to take place over a prolonged period of time and a laboratory ship had been found unsatisfactory at Operations CROSSROADS animal colonies and laboratories were built on Japtan Island at Eniwetok. These biomedical studies initiated a long and profitable cooperative program between NMRI, Los Alamos Scientific Laboratory (LASL), U.S. Naval Radiological Defense Laboratory (USNRDL), and the Division of Biology and Medicine of the U.S. Atomic Energy Commission (USAEC). The Operation GREENHOUSE bio-

medical program was a major undertaking in terms of personnel and money for each of the participating laboratories and involved the majority of the staff of the NMRI Atomic Medicine Division. The objectives of the radiobiological parts of the biomedical program were: (a) The determination of the LD₅₀ of atomic bomb gamma radiation on large animals and on mice; (b) the determination of whether the rapid dose rate was more or less effective than the ordinary dose rates used in the laboratory; (c) the relative biological effectiveness of high energy gamma radiation; (d) the relative biological effectiveness of the fission neutrons from the bombs; (e) the correlation of the pathology of radiation injury with the clinical course; (f) the quantitative hematological correlation with ultimate mortality; (g) sophisticated fundamental studies when practical in the field; and (h) a long-term study of the surviving mice to determine the late effects and, in particular, dose effect relationship with development of all types of cancer. Because of a general policy of the Department of Defense the studies on carcinogenesis were not approved for NMRI. Accordingly, it was necessary to find a satisfactory laboratory to undertake these studies on the surviving mice. Fortunately, Jacob Furth of the Biology Division, Oak Ridge, agreed to undertake this initial study. The objectives were clearly evident to the biomedical groups at NMRI, USNRDL, and LASL. In order to attain these objectives numerous planning groups were formed and ultimately G. V. LeRoy was appointed as Director of the Biomedical Program directly responsible to the Task Force Commander. Primary responsibility for different aspects of preparation were assigned to different laboratories. The development of equipment for shielding against gamma radiation was primarily a Los Alamos project. Studies on the requirements for exposure conditions of swine and dogs in the tropics were performed primarily at USNRDL. Control studies on the mortality of radiation for dogs, swine, and mice were performed at NMRI. NMRI accepted the responsibility and through the Bureau of Medicine and Surgery organized BuMed Unit One which proceeded to Eniwetok Atoll and participated in the building of laboratories and animal facilities and the breeding of extensive colonies of LAF₁ mice, swine, and dogs for the experimental studies. The subsequent operation of the colony was a joint NMRI and USNRDL project. The mouse colony produced 16,000 healthy hybrid LAF₁ mice on time for use at the specific weapons tests. The large animal colony produced 291 dogs of the appropriate size and 300 swine for the tests on schedule. The total estimated cost of this formid-

able task on a tiny island in the mid-Pacific for the fiscal years 1950 and 1951 was \$3,320,000. The later highly successful studies on the biological effects of radiation would have been impossible without the successful completion of NMRI's and USNRDL's mission in providing the required number of animals of desired age and size on specific dates.

The animal exposure equipment was designed and in part built at NMRI in order to protect animals against the thermal blast, and secondary missile effects for the study of radiation injury. This part of the program under Draeger's direction was highly successful. All equipment operated satisfactorily, attained the aims, and also protected the animals against the harsh environmental conditions of the tropical sun. Depth dose studies in tissue equivalent phantoms were carried out at NMRI under the direction of Comdr. W. H. Chambers, MC, USN. Its initial partial success formed the basis for further more refined and sophisticated studies during field tests in Nevada by Chambers and his radiation technology group. The results obtained at Operation GREENHOUSE were manifold of which perhaps the most significant attainment was the demonstration that competitive large laboratories such as NMRI, USNRDL, and LASL could work in harmonious and fruitful collaboration in the field, many thousands of miles away from base operations. The concrete scientific accomplishments were: (a) Establishment of the LD₅₀ of atomic bomb gamma radiation for mice, dogs, and swine; (b) The high dose rate of atomic bomb's gamma radiation was shown not to be significantly different in biologic effect than the ordinary dose rates used in the laboratory; (c) A first estimate of the relative biological effectiveness of the fast neutrons was obtained primarily by the Los Alamos scientific group and shown for acute effects upon spleen-thymus weight not to be in excess of 2; (d) It was clearly demonstrated that hemorrhage and infection were the major causes of death in the lethal dose range in swine and dogs; (e) The granulocyte count was shown to be a very useful sign to prognosticate the probability of survivors. Similarly, platelets were also found to have a clear prognostic value; (f) The role of the thrombocytopenia was clearly demonstrated as being of major importance in determination of the cause of radiation bleeding; (g) With the clear establishment of the major causes of death in the lethal dose range, studies on therapy of radiation injury were clearly pinpointed to evaluation of the role of platelet transfusions, leukocyte transfusions, and antibiotics.

Prior to concentration of efforts for Operation

GREENHOUSE by the Hematology Division, the problem of radiation hemorrhage was attacked. It had been published by others elsewhere and widely accepted that radiation hemorrhage was primarily due to "heparinemia" and secondarily due to thrombopenia. Whereas this concept was accepted at Operation **CROSSROADS** further studies at NMRI led to the belief that "heparinemia" rarely if ever developed in the irradiated dog. Since other NATO member nations were contemplating stockpiling of antiheparin drugs, a comprehensive attack on heparinemia was commenced. In this study an officer of the Royal Army Medical Corps participated, Maj. R. T. Lundie, in part along with G. V. LeRoy. The heparinemia concept was shown to be incorrect and antiheparin agents to be of no clinical value in dogs. Thus efforts on the study of the thrombopenia were intensified.

In 1949 it was recognized that a reliable method for platelet counting was needed before one could study reliably the relationship of platelets to bleeding. In conjunction with Brecher of NIH, a method for the enumeration of platelets involving dark phase contrast microscopy and a new anticoagulant was developed which has become a widespread platelet counting method throughout the world today.

While the bulk of the radiobiology staff of NMRI was at Eniwetok for Operation **GREENHOUSE**, a small part of the Hematology Division continued studies on the nature of radiation hemorrhage. It was clearly established that the tendency to bleed in irradiated animals was correlated with the thrombocytopenia and that all *in vitro* coagulation tests could be brought to normality by the addition of separated platelets.

Upon return of the Hematology Division to NMRI and upon completion of the Operation **GREENHOUSE** reports, the endeavors were aimed at establishing an effective therapy of radiation injury. It appeared logical that platelet transfusion, leukocyte transfusions, and antibiotics would be effective against radiation injury. The first aim was the development of methods for platelet transfusions. First, and again in conjunction with Brecher of NIH, a method for the satisfactory separation of platelets was accomplished. Parenthetically, this method with minor changes is still in use in clinical medicine for platelet transfusions today. Second, the effectiveness of transfusions of fresh platelets into irradiated animals at levels of radiation known to produce 100 percent bleeding was evaluated. It was conclusively demonstrated that platelet transfusions would prevent the development of bleeding in animals provided a platelet count be main-

tained above about 50,000 per cubic millimeter. Furthermore it was shown that platelet transfusions could stop bleeding that had already commenced. This was the first clear-cut demonstration of the unequivocal role of the platelet in the pathogenesis and the prevention of radiation bleeding.

During this same time period collaborative studies with K. M. Brinkhous, University of North Carolina, demonstrated that antihemophilic factor (AHF) was not involved in radiation hemorrhage thus showing in a basic study that AHF is not produced by lymphocytic tissues since the studies were performed while the lymphocytic tissue was aplastic. Studies in collaboration with L. M. Tocantins, Jefferson Medical College, on plasma antithromboplastin were commenced and shown to be increased.

Next, methods for the separation of granulocytes from fresh canine blood were also developed in collaboration with Brecher, NIH. It was shown that the transfusion of freshly separated granulocytes into irradiated dogs would reverse the histologic picture. The transfused granulocytes migrated to sites of infection and successfully prevented the widespread dissemination of bacteria. However, the animals would then die from extensive hemorrhage. The studies on platelet and granulocyte transfusions clearly demonstrated the essential role of these circulating cellular bodies in the pathogenesis of radiation hemorrhage and infection but unfortunately were not successful therapeutically because the levels of irradiation were inadequate to completely suppress the immune responses and after 2 to 3 weeks antiplatelet and antigranulocyte substances were produced making it impossible to continue to maintain satisfactory levels of platelets or granulocytes; thus the animals died. Preliminary studies on the combined use of fresh blood and antibiotics suggested that these would be of value in increasing the survival rate of otherwise fatally irradiated animals. This has subsequently been thoroughly demonstrated in many other laboratories.

The studies performed elsewhere had shown that the original Jacobsen (University of Chicago) concept of humoral protection against irradiation by splenic suspensions was not correct and that bone marrow or spleen cell suspensions protected by transplantation of a stem cell that repopulated the depleted bone marrow. At this time an extension of the collaborative work with NIH involved a study of the influence of parabiosis upon survival of irradiated rats. Parabiosis was initiated before irradiation and only one parabiont was given a fatal dose while the other was lead shielded. The protection was dramatic and

significantly proved that the protective cell or "humor" moved through the blood. Subsequent studies elsewhere conclusively proved the protection to arise from stem cells that can be concentrated from normal blood. The surviving parabiotic protected rats were studied. These animals had been protected from an otherwise fatal dose. A striking induction of cancer other than leukemia was observed.

In order to study further the biological effects of gamma radiation under laboratory-controlled conditions, the Atomic Medical Division under Dracger, designed and completed in 1952 a 1000 curie cobalt-60 irradiator. This source was unique in that an animal as large as a swine could be exposed to uniform gamma rays from a 4 π solid angle. Installed in a specially-built structure, the cobalt was transferred between two shielded rooms to form a cylindrical pattern around the exposure chamber. Two years later, the cobalt-60 was replaced to increase the source to 2500 curies. Many biological specimens, animals, materials, and clinical patients have been exposed in this gamma ray generator.

In continuing field tests that were being planned for Nevada there were evidently two things that needed to be studied further. First, the problems of dosimetry initiated at Operation GREENHOUSE by the NMRI group under Chambers needed to be extended and confirmed. In Operation BUSTER the Radiation Technology Division clearly established the depth dose curve of atomic bomb gamma radiation in tissue equivalent phantoms. In laboratory studies it was clearly shown that this depth dose pattern could be simulated by the 10-Mev betatron at the Naval Ordnance Laboratory (NOL). At Operation UPSHOT-KNOTHOLE in 1953 the atomic bomb gamma radiation and depth dose from fallout was studied by NMRI's Radiation Technology Division. This now classic picture of the flat depth dose curve in a fallout field was established and is commonplace in texts today. This is of particular significance because at the time of Operation UPSHOT-KNOTHOLE there was no clear appreciation that fallout was to become later a significant hazard to human beings since the accident of Operation CASTLE in 1954 had not yet happened. It is of interest to record the conclusions of the Radiation Technology Group in 1953. "The depth dose curve of the high energy component in the case of a fallout area 3.5 miles from ground zero shows a more uniform distribution of dose throughout the phantom than does the depth dose curve obtained from the initial radiation. Therefore, the effects of a given dose of radiation from a residual field could

be more serious than those from the initial radiation because of (a) the relative uniformity of the field of radiation which produces a more uniform dose throughout a man's body, and (b) the additional presence of the readily absorbed radiation." In addition to the establishment of the flat depth dose curve from a fallout field beta/gamma ratios were also presented. The preceding is a distinct example of the forward thinking in planning and making physical measurements of biological importance before the establishment of a real biological problem by experience or accident.

In Operation TUMBLER-SNAPPER there was again a fruitful collaboration between NMRI and USNRDL aimed towards establishing firmly the relative biological effectiveness of the neutron component of fission bombs. This was successfully accomplished showing the RBE to be approximately 1.6. In addition, for the first time, the peculiar neutron induced 3- to 4-day death of mice, was established. In addition, during Operation TUMBLER-SNAPPER biological studies on mice demonstrated the presence of an as yet unknown high flux of neutrons that could be a casualty producing agent to troops in the field. This was shown prior to physical measurements and computations in conjunction with the testing of this experimental tactical weapon. This emphasized the significant contribution of biological studies to the understanding of weapons effects. The mouse is a superb integrating radiation dosimeter under certain conditions.

In early 1952 the USAEC was planning a series of experimental and test explosions in which a major effort would be devoted to making studies pertinent to civil defense. The USAEC requested the Department of the Navy to permit Comdr. E. P. Cronkite, MC, USN, of NMRI to be the program director for the biological programs and to organize a collaborative study between NMRI, USNRDL, Oak Ridge National Laboratory, LASL, Lovelace Foundation, and various universities that desired to participate in basic scientific radiobiologic studies and in the evaluation of the AEC prototype shelters in respect to protection against blast, thermal, and ionizing radiation. A comprehensive program of basic and applied nature was formulated and satisfactorily executed and reported. The radiobiologic studies performed jointly by NMRI and USNRDL showed that the prototype shelters were exceedingly good shields against gamma rays and neutrons. The other objectives of field testing listed earlier with the exception of the depth dose pattern of fast fission neutrons were repeated with the same re-

sults. This field atomic bomb study clearly proved the feasibility of civilian laboratories and university scientists working under a military direction in relative harmony and with definite accomplishments.

Before and during Operation UPHOT-KNOT-HOLE the Oak Ridge group had developed a method of measuring neutron flux and energy by fission foil detectors. In 1955, utilizing the NMRI gamma detectors and the Oak Ridge fission foil detectors, an NMRI group under G. Imirie and the Brookhaven National Laboratory group under V. P. Bond studied the comparative neutron and gamma depth dose patterns. These studies were highly successful and proved that gamma and neutron radiation cannot be added rad for rad, corrected for RBE in thin foils in air to a meaningful dose because the neutron depth dose curve falls off much more rapidly than gamma depth dose curve, thus indicating that per rad fast neutrons probably are not as lethal as penetrating gamma rays.

Operation CASTLE (1954) was designed as a full scale test of thermonuclear devices. There was no planned biological program by NMRI. On 1 March 1954, following the detonation of a 15 megaton experimental device at ground level an unexpected change in the wind pattern resulted in the deposition of large amounts of fallout upon atolls inhabited by the Marshallese, American servicemen operating a weather station, upon the task force, and upon a Japanese fishing vessel, the Lucky Dragon.

The task force was able to wash down satisfactorily and personnel aboard received no significant radiation injury. However the Marshallese and American servicemen were exposed to potentially dangerous amounts of radiation from the fallout before they were evacuated by plane and ship.¹

The task force commander requested assistance from the USAEC for care and study of the irradiated individuals. The Director of the Division of Biology and Medicine of the USAEC, J. Bugher, turned to the NMRI and requested through the Surgeon General that Cronkite, be appointed as officer in charge and that he organize a medical team from the experienced personnel of the U.S. Navy. This study became again a joint NMRI-USNRDL project with Bond as assistant project officer representing USNRDL. Within 48 hours a team was assembled consisting of expe-

rienced M.D.'s, Ph. D.'s, radiation physicists, and technicians. The equipment was crated and airlifted to Kwajalein Naval Air Station to which the individuals had been evacuated. The clinical and hematological studies performed upon these individuals clearly established the symptomatology in human beings after an exposure of radiation approximately at the level of 175 rad. The sequence of events in the development of beta skin burns was documented and is now a classic study. The development of granulocytopenia, lymphopenia, and thrombocytopenia was clearly established and shown to be significantly different from that in experimental animals after comparable doses of radiation. The conservative management long recommended by the NMRI group was clearly shown to be the treatment of choice. The cardinal principles of management of radiation injury were established as meticulous history and clinical examination with attention to preexisting chronic infections, laboratory studies to estimate degree of bone marrow suppression and to avoid all therapy until something is clinically indicated. The exposed individuals experienced a severe epidemic of upper respiratory infection which also involved the staff and nonirradiated individuals thus demonstrating that prophylactic treatment of irradiated human beings by antibiotics is not necessary.

The conservative clinical management of the above individuals, in face of pressure to institute transfusions and antibiotic prophylaxis, as the granulocyte and platelet counts continued to fall, resulted in the now generally accepted policy of observe and wait for clinical indications for treatment that has subsequently been followed in most later radiation accidents.

In addition to the above clinical studies an opportunity for the study of internal contamination existed. The exposed people had lived, breathed, and eaten in a highly contaminated environment for over 48 hours. The degree of internal contamination of the people and animals was measured primarily by the USNRDL group. The studies pointed out, under the conditions that existed (breathing, ingesting, and living in a contaminated area) that the magnitude of exposure from external radiation greatly outweighed the magnitude of the internal contamination. In fact the former might well reach lethal levels whereas the latter by itself would produce little if any injury.

The body burdens of the various radionuclides were established and the studies were begun on biological turnover rats. The studies were continued by Brookhaven National Laboratories.

In 1955 and 1956 there were continuing field

¹ The following exposure groups were to be followed:

- a. 28 American servicemen about 50 rads.
- b. 64 Marshallese about 175 rads.
- c. 18 Marshallese about 70 rads.
- d. 157 Marshallese about 20 rads.

studies in Nevada in which the Radiation Technology and Pathology Divisions participated to complete the details on various of the objectives laid down following Operations CROSSROADS and GREENHOUSE.

Upon the establishment of the International Moratorium for testing of nuclear weapons experimental field work obviously terminated. The remaining problems of military importance in respect to radiobiology and radiation dosimetry clearly needed sophisticated experimental tools not available at NMRI. For this purpose radiation facilities (cyclotron and other devices) were approved for installation at USNRDL and a new laboratory was conceived, the Armed Forces Radiobiology Research Institute (AFRRI). The new laboratory would have not only a "flash" reactor but also high intensity gamma generators which could mimic the neutron gamma spectra of different nuclear weapons and thus evaluate their effectiveness as radiation antipersonnel weapons.

The serious scientific study of radiation effects upon

man and mammals for the naval service commenced at NMRI in 1945. The radiation biology program consisted of basic studies on radiation, effects on mammals, radiation dosimetry, and the management of radiation injury. The laboratory program was always dovetailed with field studies at the Nevada and Pacific Proving Grounds aimed at the solution of military problems. The coherent laboratory and field research program attained its fullest appreciation just when the Navy, through NMRI, was requested to undertake the scientific direction of biomedical programs of Operation UPSHOT-KNOTHOLE for the Civil Effects Groups of the USAEC and second when the Navy again through NMRI was requested to take the responsibility for the care and study of human beings accidentally exposed to fallout radiation at the Pacific Proving Grounds. With the establishment of AFRRI the radiobiological program of NMRI was phased out logically and responsibility for its continuation assumed by AFRRI and USNRDL.

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Regina E. Hunt