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3 MAY 1950



From: Commander, San Francisco Naval Shipyard  
 To: Chief of Naval Research, Navy Department, Washington 25, D. C.  
 Via: Chief, Bureau of Ships (Code 348)

Subj: Proposal for research project; submission of

Encl: (1) Research proposal - "The Relationship Between Radiation Damage and the Immune State"

1. A proposal for conduct of research on "The Relationship Between Radiation Damage and the Immune State" jointly by the Naval Radiological Defense Laboratory (NRDL) at this Shipyard and the Naval Biological Laboratory (NBL) at the Oakland Naval Supply Depot is forwarded herewith as enclosure (1).

2. Initial studies relating to the proposed program have been under way for approximately one year as a joint endeavor conducted by personnel of NRDL under the auspices of the Bureau of Medicine and Surgery, and by personnel of ONR Task 5 and NAMRU #1 operating under NA projects 134096 and 134000 at the University of California at Berkeley. These initial studies have indicated the extent and magnitude of the problems involved in altered host resistance following exposure to ionizing radiation. Preliminary studies have shown that transmission of disease is greatly accelerated in an irradiated population, and have indicated that present Public Health Measures may not only be inadequate but may be harmful under such conditions. The elucidation of these findings is considered to be of the utmost importance in connection with radiological defense.

3. The portion of the program under prime cognizance of the Naval Radiological Defense Laboratory is at present implemented chiefly by Naval personnel. A continuation of the program during the Fiscal Year 1951 at a somewhat increased level is indicated as desirable from the results of initial investigations. The detailed outline of that portion of the joint project proposed for accomplishment at NRDL is set forth in



Contains no U.S. Navy Classified Information

IAW OPNAVINST 5513.16

Signature: James H. [unclear] CAPT USN

Code: N 3120 Date: 8 Feb 50

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enclosure (1). It is understood that the details of the complementary portion of the joint investigations proposed for accomplishment by personnel of the Naval Biological Laboratory are already in the hands of the Office of Naval Research.

B. E. MANSEAU

J. J. FEE  
By direction

Copy to:  
BuMed, Code 74  
ONR, San Francisco  
NBL

[REDACTED]

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NAVAL  
RADIOLOGICAL DEFENSE  
LABORATORY

San Francisco Naval Shipyard  
San Francisco 24, California

Proposal for Research on  
THE RELATIONSHIP BETWEEN RADIATION DAMAGE AND THE IMMUNE  
STATE.

To be Submitted to  
THE OFFICE OF NAVAL RESEARCH

Victor P. Bond, M. D.  
Principal Investigator

26 April 1950

ENCLOSURE (1)

# THE RELATIONSHIP BETWEEN RADIATION DAMAGE AND THE IMMUNE STATE.

## I. General Statement.

This protocol represents a proposal for a collaborative research project in the fields of Radiobiology, Bacteriology, and Immunology. It is to be pursued jointly by personnel of the Naval Radiological Defense Laboratory (NRDL) at the San Francisco Naval Shipyard and by personnel of the Naval Biological Laboratory (NBL) at the Oakland Naval Supply Depot, the latter under the Office of Naval Research Contract with the Department of Bacteriology, University of California, Berkeley. For reasons of completeness and clarity the overall program is presented in this proposal, and those portions of the project to be carried out primarily by the Naval Radiological Defense Laboratory are pointed out under section V below. The cost appraisal given herein includes only the expenses for that portion of the program undertaken by the Naval Radiological Defense Laboratory. The cost of the program to the Naval Biological Laboratory will be given in a parallel proposal submitted by that Laboratory.

The major phases of the proposed investigation can be represented briefly as follows:

1. The reaction of the irradiated host to vaccines; mechanisms involved. These studies are to involve serological, pathological, and hematological techniques as required.
2. The reaction of the irradiated host to vaccines; response with different hosts and vaccines; variation of response with changes in host and vaccine.
3. The effects of irradiation on antibody production, quantitative studies.
4. Application of irradiation to studies in experimental epidemiology.
5. The evaluation of promising therapeutic agents as applicable in the course of the above studies.

## II. Background.

The importance of altered resistance to infection in the irradiated host was emphasized following the atomic bomb explosions at Hiroshima and Nagasaki, when it became apparent that infection played a significant role in deaths among casualties resulting from ionizing radiation (1). The terminal event in many of the victims was an overwhelming infection, even in cases when bone marrow function apparently was returning to normal.

The interference of radiation with leucocyte production is well known, and an alteration of the activity of the fixed and wandering reticulo-endothelial cells has been reported (2, 3, 4). Suppression of the Shwartzman phenomenon following irradiation has also been reported (5), indicating perhaps an alteration in local immune processes.

Several investigators have concluded that while whole body irradiation delivered prior to antigen administration significantly inhibits antibody production, irradiation after antigen administration produces little effect (6, 7, 9). Recent data of H.I. Kohn (8) indicate further that the irradiation - antigen administration time relationships are of great importance. By determining serially in the rat the antibody titer at a number of times following antigen administration, he obtained marked changes in the pattern of antibody response even when irradiation followed antigen administration.

Since it is generally held by radiobiologists that infection plays a paramount role in producing morbidity and mortality following exposure to ionizing radiation, it is of utmost importance that the fragmentary data bearing on the problem be extended, and that mechanisms of the change be investigated.

As an initial step it is necessary to establish quantitatively the degree to which irradiation alters the resistance to infection, and the time relationships pertaining between the two factors. This should be augmented by investigations into possible mechanisms responsible for altered susceptibility to infection. Studies on the effect of irradiation on actively and passively induced immunity require investigation, with the end in view of determining the efficacy of established prophylactic practices in public health if irradiation becomes a factor. An evaluation of the extent to which transmission of disease is altered in the irradiated population is of great importance.

#### 111. Results of Experimentation to Date.

During the past year investigations on a limited scale have been undertaken by personnel of the Naval Radiological Defense Laboratory (provided by the Bureau of Medicine and Surgery), and by personnel of the NAMRU #1 and ONR Task 5 (provided under projects NR 134096 and NR 134004, contracts N6 onr 111, Task Order V, and contract N7 onr 29527, both with the Department of Bacteriology, the University of California at Berkeley). Results to date have been presented both in progress reports of the Naval Radiological Defense Laboratory and in the Status Reports of the Berkeley Group, and may be summarized as follows:

- a. Homogeneous x-irradiation of mice was obtained by the following method: Seven-to-nine-week-old Namru strain mice were exposed in lusteroid centrifuge cones fastened to the surface of a board which

rotated an appropriate distance from a radiation source in order to eliminate non-homogenetics in the radiation field. Two studies on irradiation mortality yielded LD50 values of approximately 665 r and 550 r. Male and female animals showed similar LD50 values. Using these results, a value of 350 r whole body radiation was selected and found to be sublethal for 4-5 and 7-9-week-old-mice, regardless of sex. In all cases normal animals, chosen at random from a common pool, were subjected to identical procedures except actual exposure to irradiation.

- b. Irradiated mice, when exposed to air-borne streptococci, were found to be about three times more susceptible to infection than the normal controls. This effect was observed at intervals from 3 to 30 days after x-irradiation but was no longer present 50 days after exposure. A limited number of trials indicate that increased sensitivity is also a function of x-ray dose. The dose rate dependence and energy dependence of the phenomenon have not as yet been investigated.
- c. Since results under (b) above depend upon calculations involving respiratory capacity, this factor was evaluated in normal and irradiated mice with the aid of  $P^{32}$  tagged bacteria given via the respiratory route.

No difference could be noted; in addition, relative percent distribution of  $P^{32}$  was independent of the duration of the post-irradiation period.

- d. Experiments dealing with simultaneous exposure of normal and irradiated mice to air-borne P. pestis and M. tuberculosis are currently under way.
- e. As the initial step in applying the above data to studies on immune animals, a living avirulent antigenic strain of P. pestis was administered subcutaneously according to a standard procedure for immunization to groups of 100 sublethally x-irradiated mice 6 days after their exposure. Ninety-one irradiated mice (as opposed to 17 normals) succumbed, apparently as a result of the vaccination.

In three subsequent trials, this observation has been confirmed with two different living avirulent strains, with a heat treated strain whose viability was markedly reduced, and with a Seitz filtrate of a living avirulent strain, but apparently not with one of the above living strains administered under somewhat different conditions 35 days following irradiation. Experiments to confirm and extend these observations are under way. Nothing is known at present concerning the specificity of this phenomenon to P. pestis or the

possibility of its presenting a more general application to the use of living vaccines following exposure to radiation.

- f. A pulmonary infection in the rat colony at NRDL was taken advantage of to study the effects of irradiation on a naturally occurring endemic infection. Results indicate that not only is the evidence of infection increased in the irradiated population, but that the combination of irradiation and infection is much more lethal than either one alone.

#### IV. Other Research Projects in Progress.

The Biological Branch of the Naval Radiological Defense Laboratory is at present carrying on an extensive program, dealing in general with the effects of ionizing radiation on mammals. Support is provided for the most part by the Bureau of Medicine and Surgery and the NEPA (Nuclear Energy for the Propulsion of Aircrafts) project.

#### V. Proposed Experimentation.

As pointed out in (I) above, the proposed program is to be prosecuted jointly by personnel for the Naval Radiological Defense Laboratory and personnel from the Naval Biological Laboratory. In the breakdown given below, the portions of the program to be performed primarily at each laboratory are indicated.

##### a. Experimentation at the Naval Radiological Defense Laboratory.

Laboratories, personnel, and equipment (including complete equipment for immunochemistry studies) will be provided by the Naval Radiological Defense Laboratory; NBL will aid in planning experiments and will provide consultation. It will also provide such culture media, biologicals etc. as may be pertinent and convenient.

##### 1. The Response of the Irradiated Host to Vaccine. Mechanism Involved.

It is proposed to investigate the phenomenon of lethal response of irradiated hosts to avirulent or dead vaccines as reported under (III-e) above. Avirulent P. pestis will be employed, and fractionation of the bacterium will be used to further elucidate the observed findings. This strain yields antigens amenable to use in quantitative antibody titer techniques such as those of Heidelberger, which may aid in investigating the abnormal response observed. It is hoped that this study may aid not only in explaining this response, but that it may shed light on the nature of the basic alteration in the immune state that is produced

by irradiation.

Hematological and pathological studies on irradiated animals dying from vaccine administration will be carried out.

2. The Effects of Irradiation on Antibody Production, Quantitative Studies.

The same antigen used in (1) will be used to investigate, by means of Heidelberger's technique, antibody response as affected by irradiation given at various times relative to antigen administration. It is hoped that the role of the antibody suppression in the altered immunity observed following irradiation will be clarified by these investigations. The effects of radiation on the passively immunized animal, and the ability of an animal, actively immunized previously but with minimal blood titer remaining, to respond after irradiation to a challenge dose of antigen will be investigated.

Hematologic data specifically relating to the peripheral leukocyte and bone marrow responses of irradiated-infected animals will be obtained as indicated. The hematological response of "Immunized" animals that have been irradiated and infected will be studied. The minimum dose that will allow a peripheral WBC response to bacterial infection will be determined. The WBC will be followed in irradiated animals in which a leukopenia has previously been induced by means of benzene or a virus infection. Pathology relating to these infection studies will be carried out as indicated.

3. The Evaluation of Promising Therapeutic Agents as Applicable in the Course of the Above Studies.

In view of the importance of infection in irradiation damage and the lack of effective therapeutic agents, the Naval Radiological Defense Laboratory will investigate the efficacy of any promising therapeutic drugs or regimens.

b. Experimentation at the Naval Biological Laboratory.

Laboratories, personnel, and equipment will be provided by NBL, NRDL will aid in planning experiments and will provide consultation. It will also provide irradiation facilities, and will be responsible for all matters pertaining to irradiation aspects of the experiments.

1. The Reaction of the Irradiated Host to Vaccines; Response with Different Hosts and Vaccines; Variation in Response with Changes in Host and Vaccine.

It is proposed that during the fiscal year 1951 a good deal of emphasis be placed upon the elucidation of the finding reported in (III-e) above, initially using BCG, avirulent P. pestis, E. coli and cowpox virus. It is anticipated that, whether the phenomenon proves to be specific or general in application, it will be necessary eventually to evaluate variation in host response as a function of pre-history and condition of vaccine; age, sex and species of host; type and degree of radiation; and time relationship between irradiation and vaccination.

It should be pointed out that the present vaccine of choice for P. pestis is an avirulent lyophilized living culture.

2. Application of Irradiation to Studies in Experimental Epidemiology.

Another aim of the study is the use of irradiated animals to increase the susceptibility of the host to disease and thereby, it is hoped, to increase the transmission rate. This portion of the project is of prime interest to the Naval Radiological Defense Laboratory as it offers opportunity to evaluate the response of individual hosts, and of populations following irradiation and exposure to respiratory infection. The program will include studies on streptococcal infection, M. tuberculosis, K. pneumoniae, and P. pestis infections.

VI. Scientific Personnel.

1. Victor P. Bond, M. D., Principal Investigator at the Naval Radiological Defense Laboratory. A.B., University of California at Berkeley, 1943; M.D., University of California at San Francisco, 1945. One and one half years training in radiobiology at the Donner Laboratory of Medical Physics, University of California, Berkeley. One and one half years research experience at the Naval Radiological Defense Laboratory. A portion of the work accomplished during this time has been reported as NRDL publications AD(92)B, AD-199(B), and AD-198X; other work is in preparation for publication. Advancement to candidacy for Ph.D. in Medical Physics has been granted Dr. Bond by the University of California at Berkeley.

Born in California, 30 November, 1919  
Available at NRDL; Valencia 4-1900, Ext. 2672.  
Will devote one half time to the project.

2. I. L. Shechmeister, Ph.D., Principal Investigator at the University of California at Berkeley. A.B., University of California at Berkeley in 1934; M.A., in Bacteriology obtained at the University of California at Berkeley, 1935; Ph.D., obtained at the University of California at Berkeley, 1949. Teaching experience in bacteriology, 4 to 5 years. Lecturer in Public Health (Biometrics and Epidemiology). Dr. Shechmeister has had 15 years research experience of which 12 years have been spent in independent research. He has published 15 papers in Bacteriology. Work relating to present project is now in preparation for publication.

Born in Latvia, 1913. Naturalized in 1939.

Available at WASHU #1; Thornwall 3-3445.  
Will spend 3/4 time on the project.

3. Myron S. Silverman, A.B., Bacteriology, Cornell, 1937; M.S., Bacteriology, Cornell, 1938; Ph.D., to be taken June 1950, University of California at Berkeley. Two years teaching experience in bacteriology; 8 years research experience. Recent experience at the University of California under Dr. Sanford Elberg was concerned with the immunochemistry of P. pestis, involving fractionation, purification and characterization of antigens, and quantitative studies of antigen - antibody reactions.

Born in New York, 1915.

Available through WRDL, Valencia 4-1900, Ext. 2672.

Will spend full time on the project.

## VII. Cost Estimate.

This estimate includes only that portion of the project to be carried out primarily at NRDL. Estimates are based on cost for one year, the program is to begin 1 July 1950.

### Salaries

Principal Investigator (Victor P. Bond, M.D., USN) No charge	
Principal Bacteriologist (Myron S. Silverman, Ph.D.), GS-9	\$4,600.00
Bacteriologist (to be obtained) GS-7	3,825.00
Technician (Hematology, Tissue preparation) GS-5	3,100.00
Technician (Serology), U. S. Navy	No charge
Animal Caretaker (Laborer)	2,919.00
Pathologist (1/5 of full time)	1,500.00

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TOTAL \$15,944.00

Overhead (15% of salaries) \$ 2,390.00

### Equipment

Animals (Rabbits, Guinea pigs, rats)	\$ 1,500.00
Animal food	500.00
Miscellaneous supplies (Drugs, special media etc.)	300.00
Miscellaneous small equipment (magnetic stirrer, Seitz filters etc.)	750.00

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TOTAL \$ 3,050.00

Irradiation facilities, 100 hours  
at \$3.00 per hour 300.00

### Travel

One trip to Camp Dietrich) 350.00

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TOTAL \$ 650.00

GRAND TOTAL \$22,034.00

## BIBLIOGRAPHY

1. Brues, A. M. et al. General Report. Atomic Bomb Casualty Commission. National Research Council, January 1947.
2. Crom, Sr. A. Studies on the effects of roentgen rays upon the intestinal epithelium and upon the reticulo - endothelial cells of the liver and spleen. Acta Radiologica 16:641, 1935.
3. Glenn, J.C., Jr. Further studies on the influence of x-rays on the phagocytic indicer of healthy rabbits. J. Immun. 53:95, 1946.
4. Pohle, E. .. The effects of roentgen rays on the reticulo-endothelial system. Am.J. Roent. 22:439, 1929.
5. Becker, R.M. Suppression of the Shwartzman phenomenon by nitrogen mustard, Benzol, and x-ray irradiation Proc. Soc. Exper. Biol. and Med. 69:247, 1948.
6. Craddock, C. G. and Lawrence, J. S. The effect of irradiation on antibody formation in rabbits. J. of Immun. 60:241, 1948.
7. Haktoen, L. Further observations on the effects of roentgenization and splenectomy on antibody production. J.Infect. Dis. 27:23, 1920.
8. Kohn, H.I. The effect of total-body x-irradiation upon the hemolysis response of the rat to sheep erythrocytes. ORNL 391, October 1949.
9. Taliaferro, W. H. and Taliaferro, L.G. The effects of x-rays on immunity. National Nuclear Energy Series, Vol. 22B, Div. IV, paper 1.11a.
10. Heidelberger, M. & Kendall, F. E. Jour. Exper. Med. Vol. 65:647, 1937.