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REVIEW OF THE BIO-MEDICAL PROGRAM IN
OPERATION GREENHOUSE
BY: DICK KOOGLE 6-8-81

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I General Considerations:

A. Program: The Bio-medical program is well-conceived and well planned. It has minimal objectives of a practical nature for 1) determining biologically the quality and quantity of the radiations from a standard atomic explosion, and 2) for obtaining information of civil defense and military value. It has taken into consideration the indefinable variables of biological experimentation in the field and is doing everything possible in the way of controlling these tests so that the difficulties inherent in evaluating the results will be minimized. It has consistently adhered (except for one minor concession to the Air Forces, and another to the Surgeon General's office) to the basic policy of limiting the types of experimentation to these of a well-tested and well-understood nature in order that the test may serve to correlate what happens to animals exposed to an atomic explosion with what is being studied in the laboratory. The biostatistical aspects of the program may be considered to be representative of the thought and planning which have gone into this operation. Even such factors as pre-test randomization of the animals to be sacrificed serially or to be shipped back to the States for long term study have been taken into consideration.

B. Procurement and Logistics: well under control. The animal colonies on Japtan are thriving, the large animals have been dewormed and are in good condition, the effects of trans-oceanic flight on mouse colonies have been studied, etc.. The animal stations have been procured and appear to have been subjected to adequate performance tests.

C. Personnel: Those whom I know are competent and well qualified for their jobs. The persons in charge of the Bio-medical program are scientifically competent and have a good practical understanding of logistics and organizational problems.

D. Operations: The operational aspects of the program have been well thought out and will be rehearsed thoroughly before shot time. The scientific personnel will be kept out of the field and will be responsible only for placing the biological materials in insulating liners which will then be distributed at the proper stations and collected by competent military teams. There will be five 5-man recovery teams, each with a monitor from General Cooney's outfit that will be responsible for placing and collecting the liners. Only two of these teams will go into areas which will be radioactive and the other three will be used for collecting Fearse's material on non-active islands. They will serve as reserve teams from the point of view of radiation exposure. The teams will be allowed to take an average of one roentgen of gamma radiation per man (but Le Roy believes that this dosage can be increased to 3 r if necessary). The monitors will act in an advisory capacity, but will report to a chief monitor (Captain Haight) who will be with the beach master and will have authority to order evacuation. Le Roy will be in a boat nearby and will be in direct communication with the teams and with the beach master. The problem of recovery of biological material should not prove to be difficult. The station design provides for alternate methods of recovery of material in case the usual methods of evacuation cannot be used. The radiation hazards for this type of an explosion can be estimated with reasonable accuracy and should not be serious--150 mr per hour

is expected to be the highest radiation intensity which the teams will encounter.

It will take about 4-5 hours to distribute the animals before the shot and about an equal time to return them to the laboratories. Immediately after the shot, a helicopter will go into the area, give the all-clear signal, collect a few neutron indicators and do minor repairs concerned with air flow into stations. A special crew will arrive by speedboat on the island within an hour after the shot, will be prepared to repair stations and to obtain the first two pairs of pigs and dogs for serial sacrifice. These animals will be returned to the laboratories within three hours after shot time.

E. Over-all Plan of the Operation: will be given verbally.

II Biological Materials to be used and Methods of Display:

A. Types of Materials: The biological materials to be displayed differ for the two tests in the following way:

Test 1. 1. Biological Dosimeters

- a. Corn in packets (for high flux neutrons)
- b. Tradescantia (inflorescences and plants to be used primarily for lower doses of neutrons)
- c. Mice (thymus-spleen weight change, to be used for both gamma rays and neutrons.)

2. Mammals (Survival vs. distance)

- a. mice
 1. L.D. curves (30 day)
 2. long term effects
 - a. gamma ray survivors
 - b. neutron survivors
- b. pigs
- c. dogs

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3. Mammals (Special Studies)
 - a. dogs
 1. serial sacrifice
 2. hematology (? also pigs)
 4. Mammals (Thermal effects vs. distance)
 - a. dogs and pigs (1:4 ratio)
 1. three tests per animal (time versus effect)
 2. three tests per animal (wave length vs. effect)
 5. Radiation Measurements (by Bio-medical group)
 - a. Radiant Energy (to be measured by N.R.L.)
 1. total integrated dose at each station to be measured by Fears
 - b. Ionizing Radiation
 1. Man-sized laminated masonite phantoms: Each will contain film and ion chambers to give an idea of the homogeneity of the incident radiation and the amount of scattered radiation. A pair of these phantoms will be placed at each large animal station (with same amount of shielding); others will be placed at closer and further points; and some will be placed in standard army tanks.

2. Lucite spheres ranging from 5 to 30 cm. in diameter. A graduated series of spheres will be suspended at each station (? large and small animal station). They contain film packets and ion chambers (high saturation) and are designed to give the radiation doses in air.
3. Mouse Phantoms: Mouse sized lucite blocks containing film to be placed at each mouse station. .
4. Other measurements of neutron and gamma ray intensity and spectrum will be made by N. R. L. and Los Alamos group.

Test 2. Studies by Bio-medical group limited to the following:

1. biological dosimetry
2. mouse survival vs. distance
3. thermal effects in large animals
4. ionizing and thermal radiation measurements

B. Exposure Stations: Except for the biological materials in the drone planes, all biological stations are on the ground (or on floats) along one of several nearby radii. The stations are of different types and are designed to withstand varying amounts of blast and to shield against different types and amounts of ionizing radiation. All stations except those in the planes will have individual cells for the animals as well as a continuous record of the temperature and pressure, automatic airflow (which

shuts off temporarily after the shot to exclude possible contamination by fission products.)

1. Ground Stations:

Type 1: To house packets of corn. Don't know details of design but will be very close to tower.

Type 2: Hemisphere type stations for neutron dosimetry. Has great strength and shields out almost all gamma rays by means of 6 in. layer of lead painted on the outside with cadmium.

Only neutrons above thermal energies will enter this station. The attenuation of the neutrons will constitute less than 20 per cent of total. There will be eight of these stations for neutron dosimetry containing mice, tradescantia, mouse phantoms, personnel and neutron dosimeters; eight additional stations will contain mice for neutron survival studies.

Type 3: Cylinders for gamma ray exposures of mice and tradescantia. Twenty-nine such stations farther away from tower than type 2. Will contain 260 mice each for gamma ray dosimetry, lethal dose and gamma ray survival studies.

Type 4: Large animal containers shielded from blast effects for gamma ray mortality

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and serial section studies.

Type 5: Large animal stations for thermal effect studies. Animals are anesthetized and held in place against quartz covered exposure ports (the quartz windows will shield against sand blast effects). In those ports, where time vs. effect is studied, the signal is taken from the flash by a photoelectric cell (called "bluebox", apparently very reliable signal corps equipment).

2. Drone Animal Chambers: Mice are placed in two compartments of the drone fuselage. Individual cells are not used. In one compartment, the outside air containing f.p. is admitted. In the second compartment, the air is filtered and mice and tradescantia inflorescence are used as integrating dosimeters. These designs have been tested out at Sandia. Mice withstand the lowered temperature and oxygen tension well. Tests are also being conducted on radiation effects at simulated pressure and temperature at Los Alamos.

III Detailed Plans of Individual Experiments:

A. Biological Dosimetry

1. Corn: Corn undergoes a disproportionately large number of somatic mutations when irradiated with neutrons. Tackets of kernels will be exposed to differing amounts of radiation ranging from a minimum of 15,000 gamma roentgens and 10^{15} neut/cm² to a maximum of 300,000 gamma r and 10^{15} neut. Closest station will be 300 yards and recovery will be delayed for several days. The seeds will be

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studied by Anderson of C.I.T., who made observations on the Bikini corn. Controls have been studied carefully with 250 k.v. x-rays and with water boiler neut. at Los Alamos.

2. Tradescantia: Inflorescence will be placed at close stations and plants at far stations. Dr. Conger of Oak Ridge will be in charge of this study. Smears of the pollen will be made at 21 hours and four days after exposure (the two classical times of study) and some of the scoring of the chromosome breaks will be made at Japtan. This type of study is of particular importance in the study of neutrons as the increase in breaks is not (?) linear in the case of gamma rays when the dose exceeds 250 roentgens. I presume that the neutron studies with fission, pile and cyclotron neutrons such as have been reported in the literature are being continued. The tradescantia will be placed in the hemispherical stations where they will receive an estimated 10^{10} to 10^{13} neut (largely fast) and not more than 30 gamma roentgens. They will also be placed in the cylindrical stations where they will be exposed mainly to gamma rays.
3. Mouse spleen-thymus weight decrease: This is an excellent and novel idea, but it is the least well explored of the biological tests (except for the inhalation). Carter at Los Alamos has studied this problem thoroughly with neut. and photons of different energies and has calibrated the weight decrease in these lymphoid tissues

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for different radiations. He has demonstrated excellent families of curves which show, for example, a decrease in thymic weight of 35 and 10 per cent respectively for the same doses of 250 and 1000 k.v. x-rays. The spleens are weighed immediately after autopsy of the animals, but the thymus is placed in formalin over night to permit easier separation of the fat. 1800 separate weighings will be done in paired weighing bottles for each test. He has five balances, a regular staff of seven and will add seven other helpers to his group after each test. A trial run using 250 k.v. x-rays will be conducted on Japtan on the generation of mice preceding those actually used in the first test.

B. Mouse Experiments (other than dosimetry) The F_1 hybrids of the LAF strain are being used because they give the most consistent data on mortality studies according to Cronkite and Lorenz.

1. Mortality vs. distance: This will only be determined for gamma rays. There will be thirty animals of each sex in the twenty-nine cylindrical stations, which are so placed that they will receive increasing doses of gamma rays in increments of 25 roentgens. The probable dose range which will be covered is 40-1200, but it may be 50-1400, or 80-1600 r., depending upon the bomb efficiency. I don't think these animals will be autopsied. They should be recovered in 4-5 hours. Lethal dose studies under simulated field conditions have been completed at Hunter's Point.

2. Long term effects in survivors: The life span, tumor incidence and cataract induction will be studied in surviving mice exposed both to neutrons and gamma rays. These animals will be sent by air to Furth at Oak Ridge for observation.

a. Gamma ray survivors: There will be about 200 animals at each of the 29 gamma ray stations earmarked for long term survival studies. Following the shot, they will be pooled in groups of four (covering a dose range of 100 roentgens). Not more than 4200 animals will be returned to the states. They will be accompanied by controls (? how many) which will be given equivalent doses of 250 k.v. x-rays (for each individual group, not the pooled groups of four) according to the mouse dosimetry measurements (My own opinion is that since mouse dosimetry is a rather new tool for calibration of radiation they should also have control animals given doses based on the physical measurements of what the animals received. This would allay possible controversy in the future as to the significance of the results.) The difference in life span, tumor incidence and cataract development between the animals exposed in the field and those given equivalent doses of 250 k.v. x-rays will be studied. Only 200 non-irradiated controls will accompany the exposed animals. The statisticians felt that no more were necessary since the differences in long term effects between

unexposed animals of this strain and those given various amounts of 250 k.v. x-rays have been well established.

- b. Neutron survivors: The survivors of the eight neutron groups of neutron-exposed animals (50-60 per group in hemispherical stations) will also be returned to Furth. They will be exposed to approximately the following number of rem of neutrons: 14, 40, 70, 140, 560, and 1120. I don't know how they are going to control these groups (I suppose by unexposed animals).
3. Inhalation Experiments: Groups of (?) 30 mice will be placed in the fuselage of drone planes and the outside air including f. p. will be admitted through louvres into their container. When the animals are returned to Japtan, they will be sacrificed serially. The lungs, g.i. tracts, skin and carcasses will be pooled, frozen and returned to Los Alamos for assay. The total radioactivity of the organs, and that due to molybdenum, will be determined. In this way it will be possible to differentiate between f.p. deposited on skin and subsequently swallowed from that inhaled. I don't know what kind of physical air sampling devices will be used to control this experiment other than those used to collect samples for yield chemistry. This is one of

two instances in which there has been a minor deviation from the program's basic philosophy of conducting only well understood and easily interpretable experiments. The significance of the mouse inhalation data will not be clear, but the Air Forces have been extremely eager to obtain this information which although poor, they feel is better than none.

C. Large Animal Studies:

Pigs and dogs (foxhounds) will be used. There is some concern about whether the pigs will be small enough to fit some of the containers as an error was made in the time of breeding. This together with the slight delay in the shot time has caused anxiety particularly on the part of Pearse's group as to whether the animals will not be too large at shot time. The veterinarian in charge of the animals is confident that he can hold down the weight of these animals to 70 per cent of that formerly expected without interfering with the health or nutrition of the animals. Le Roy is going to send word to Pearse as soon as he arrives and looks over the situation.

1. Ionizing Radiation:

- a. Mortality versus distance: There will be 3 stations each containing 10 dogs and 10 pigs placed at 75 roentgen intervals covering an expected range of 60 to 700 r of gamma radiation. No testing of any sort will be done on these animals. All deaths will be autopsied. Kodachrome films will be taken of pathological specimens and some of the gross organs will be embedded in plastic and saved for teaching purposes.

Lethal dose studies have been done on the same strain of pigs at N.R. D. L. with 1000 k.v. x-rays under conditions designed to simulate those at the test site. A walk-in refrigerator has been revamped to approximate the temperature, infra-red light conditions, mean humidity and minimum air flow at stations. Animals are given 4 5 hour rides before and after irradiation in closed trucks to make exposure conditions as near as possible to those in the field. Dog experiments of a comparable nature will be completed soon. No mortality studies will be done at test site.

b. Histological Studies: Thirty-two dogs will be exposed to a 30 day L.D. 100 (approximately 550 gamma roentgens). They will be sacrificed on a random basis two at a time. The times of sacrifice have not been decided definitely and will depend upon the outcome of current studies at the N.R.D. It is probable that three groups will be sacrificed during the first 24 hours and then one pair per day thereafter. Commander Tullis will be project officer in charge of these studies on pigs, while Dr. Arthur W. Eaton (Navy) will conduct the studies on the dogs. (Col. De Coursey will not participate in this work.) As far as I can tell from talking to the pathologists at Rochester this data alone should give information whereby the equivalent dose of 1000 k.v. x-rays can be determined to within 50 roentgens. These animals will also be used

for daily hematological studies mentioned below and the blood of the dogs will be saved for chemical studies.

- c. Blood Counts: To be done daily on dogs and pigs by Commander Cronkite.
- d. Blood Chemistry: 50 percent of the dog blood at autopsy will be saved. The plasma will be separated and frozen. The following studies are contemplated:

- 1. plasma fibrinogen (?? by whom)
- 2. non-protein bound iron (Chanutin)
- 3. serum potassium (Hastings)

The remainder of the plasma will be stabilized and preserved by passing through an ion exchange column (Libby, U.C.L.A.).

- 2. Thermal Radiation: There will be five stations in the first test, six in the second, each containing eleven animals (three or four pigs for every dog). Each animal will be anesthetized and held in place against the ports designed to admit non-ionizing radiations of different wave length or at different times after the explosion. Three combinations of biological measurements will be made--the first, the thermal burns versus time i.e. during the first 30 millisecond interval after the explosion and during the period thereafter; the second, the burn produced on skin gradually exposed by a sliding shutter which opens at a definite rate of speed, and third, burns produced by excluding certain components of the light spectrum, i.e. by filtering out individually the ultraviolet, the visible, and the infrared light. Each of these tests will be carried out on both pigs and

dogs and each will have its own control of a small port which admits all of the thermal radiant energy. The only studies which will be made on the burned areas are 1) description of intact lesions, supplemented by Kodachrome photography, and 2) histological examination of biopsy specimens.

Another biological test that has just been included at the request of the Surgeon General's Office is the effect of thermal radiations on small pieces of material in contact with the skin. Twelve such tests will be made on four animals in the outer stations. (This will be integrated with the material testing program being carried out by the N.D.R.L.) Each station will have an integrating calorimeter which will measure the total radiant energy. These instruments have been designed so that their records of total energy can be broken down in terms of N.R.L. data into time and spectral distribution.

The stations are placed so that a large range of energy is covered. It is estimated that the minimal energy studied during the first test will be 3.3 to 3.3 calories per sq. cm. while the maximum will be 57 to 125 cal/cm². A comparable energy range will be studied during the second test. The minimal energies are approximately those required to produce threshold effects (5.7 cal/sq. cm/sec.). These estimates of thermal energies are quite uncertain. They depend upon who makes the calculation and will certainly vary with bomb efficiency, atmospheric conditions, etc..

These studies have been well controlled by preliminary experimentation here at Rochester. 131 individual tests on

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anesthesias have been made under conditions which simulate those in the Pacific. 40 large surface burns have been produced by the scanning technic utilizing the focussed beam of a large searchlight, and 126 smaller burns have been produced with magnesium flares.

IV Impression of Value of Data which will be obtained and problems left unsolved.

I don't think that the bio-medical program could have been planned in a more direct or simple way in order to get the desired information. Some of the tests are more straightforward than others and probably have a better chance of giving good data. Thus, ^{as far as of interest} the biological dosimetry is not subject to the many unknown variables which determine for example survival studies. These tests seem to me to be excellent and should give valuable information. The lethal dose studies are sound, although unexpected variations in radiation flux (due to bomb efficiency) may interfere with the interpretation of the large animal studies which are based on a relatively few groups of animals. The mouse survival studies should give good measures of the neutron and gamma ray effects provided that some of the intangible factors of this type of study do not distort the results. The hematology and histological studies, although performed on a limited number of animals, should also give good data. The numbers of animals in each group is statistically sound, but I am enough of a biologist to feel that the sample size ^{is} ~~may be~~ on the small side. The thermal studies have been very thoroughly planned. The factors which could interfere with

the obtaining of good results are 1) poor placing of stations due to miscalculations of the thermal energies and 2) the possibility that the pigs will be too large for use. The data from mouse inhalation studies will be difficult to interpret although it may give some clue to particle size in the cloud.

At best, good data will be obtained correlating biological effects and physical measurements. This should help to solve the discrepancies in the interpretation of dosage in the Japanese and the arguments as to dose-intensity effects. If the results from the various tests should not jibe or should conflict, it may still be possible to discriminate between true and spurious results. The problems which will not be solved by this test are impossible to anticipate, but my guess is that if you are planning another test, the dose-intensity question should be considered as the most likely to be still in question. Another practical piece of information, which can be obtained in time in the laboratory but which could be determined (with limitations) at a later test is the combined effect of ionizing and thermal radiations on large animals.

Note: Donaldson will not participate in test and testing like that done at Bikini will not be carried out.