

SENSITIVE CROSS SECTION OF A BACTERIUM,
ESCHERICHIA COLI, TO FAST NEUTRONS*

by

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REVIEW OF LITERATURE

In an examination of the effects of radiation on biological systems, the physical, chemical, and biological reactions, and their correlations warrant individual consideration. According to the observations of Andrews⁽⁵⁾ and Platzman⁽⁴⁶⁾, the time required for these reactions is variable. Most of the physical events are over in about 10^{-13} seconds, the physiochemical changes are completed in 10^{-11} seconds, and chemical changes take place in 10^{-6} seconds. Thus all the original processes which are associated with the absorption of the radiation energy are completed in less than 10^{-4} seconds. In contrast with the initial action, the biological changes which are brought about by the deposition of radiant energy in biological material may take from a few minutes to 20 years to become evident. At present, much is known regarding the physical reactions of radiations in gases. Less is known concerning their interaction with liquids and only little information is known concerning their effects in solids. In biological systems, dose and observable end effects have been correlated. These studies include the effects of adding specific materials such as glutathione and cysteine^(28,29,45,53) to the material to be irradiated before, during and after irradiation, the effects of oxygen tension,^(8,18,19,20,58,59) and mixtures

of radiation^(32,34,55). The two intermediate stages, the physiochemical and the chemical are still a mystery.

Of the radiations which are believed to cause biological mutations, ultraviolet light has been the most thoroughly investigated. It has been determined that the absorption coefficient for ultraviolet radiation depends upon the molecular structure of the target molecules. Action spectra⁽¹¹⁾ studies have shown that ultraviolet radiation in the region of 2600 Å is the most effective for the production of lethal mutations in bacteria. Blum⁽¹¹⁾ demonstrated that nucleic acids have absorption spectra which nearly coincide with the lethal action spectra for microorganisms. This suggests a relationship between lethal mutations and the disruption of nucleic acids.

Studies of the killing of bacteria with monochromatic ultraviolet radiation give evidence that the effect is independent of intensity,^(32,47,65) and survival vs. dose curves obtainable by these studies are exponential. These results demonstrate that an observable effect is due to the absorption of a single quantum in a sensitive volume. The sensitive area or volume may be the volume of the gene or genes which determines the mutated characteristic^(12,22).

Although x-rays are also electromagnetic radiation, the difference in wavelength produces a difference in effect. The absorption coefficients for x-rays are found to depend

upon the atomic number of the individual atoms which lie in the path of the x-radiation⁽²⁷⁾. Since x-rays and gamma rays affect matter in the same fashion, they are discussed together in this paper.

Long wavelengths of x-rays traverse only thin sections of tissue because x-ray intensity falls off exponentially according to the equation:

$$I = I_0 e^{-ux} \quad [1]$$

where u is the sum of three attenuation coefficients due to the photoelectric effect, the Compton effect, and pair production; I_0 is the original intensity, and x , the thickness of the sample. This total attenuation coefficient is a function of the atomic number of the target material. The x-ray energy deposited in tissue may produce either ionization or excitation; but it has been determined that ionization is 10^4 times as effective as excitation in producing observable biological effects.⁽³⁴⁾ Experiments to determine true target volumes have been performed with radiations which produce clusters of ionization widely separated along the tracks of the radiations such as short wave length gamma or x-rays.

The primary effect of x-rays on living cells is upon the chromosomes and lethal effects are thought to be due to mutations in the chromosomes.^(2,16,23,31,32,33,34,54,57,61)

X-ray data on lethal mutations of the sex chromosomes of Drosophila representing gene mutations and chromosome deficiencies follow the simplest type of Poisson distribution:

$$n = n_0 e^{-bd}$$

where n is the number of genes affected, n_0 is the original number of genes, d is the x-ray dose, and b is a probability constant. Values obtained by various authors give values for the mean lethal dose to Escherichia coli by x-rays varying from 5400 r. ⁽⁶³⁾ to 5700 r. ⁽³⁸⁾ using .15 Å and 4200 to 8400 r. as the wavelength varies from .56 to 4.0 Å. ⁽⁶⁴⁾

Theoretical expressions (Klein and Nishina ⁽³⁵⁾, and Bethe and Ashkin ⁽⁹⁾) indicate that for low Z material and low energies, the linear absorption coefficient for x-rays decreases as the inverse cube of the energy. Thus the lower energy x-rays will deposit more energy per unit path length than higher energy x-rays. Biological material is composed, except for a small fraction, mainly of the elements 1^H , 6^C , 8^O , 7^N ; and the x-rays which have been used to study such material are in the low or medium energy range. Thus the fact that the mean lethal dose increases with wavelength and ion density gives credence to the theory that an ionization in a sensitive volume will cause a lethal mutation. Since it takes a greater dose to achieve the same effect with longer

x-rays, i.e. with a greater ion density, it would seem logical to assume that the extra ion density would be wasted and that only one ionization in a sensitive volume would be necessary to produce a lethal mutation.

The electrons which are scattered by x-rays cause further ionizations. They displace electrons in nearby atoms, transferring their energy to these struck electrons in the process until their energy is depleted. This same effect is observed when electrons, protons, deuterons, and alpha particles pass through biological substances. If the ejected electron is of low energy (100 ev.), its range is a few millimicrons in tissue. If this electron has enough energy, it may become a highly ionizing particle itself and will travel several atomic radii from the original track, producing ionized atoms as it goes. Approximately half of the total ionizations produced by the original particle are produced by secondary rays having energies over 100 ev. The other half is distributed among the isolated primary single ionizations and the clusters of two or three primary ionizations.

Protons and heavier particles leave relatively straight paths of ionized atoms. The specific ionization along the paths of these primary ionizing particles is a function of the initial energy of the impinging particle. If a proton

or more massive particle of moderate energy is used as the projectile, the probability is almost unity that the heavily ionizing particle will cause at least one ionization in traversing a sensitive volume.⁽⁶⁾ Thus the probability of a hit depends on the target area rather than the target volume.

The theory that deuterons ionize in area and electrons ionize in volume, has been used extensively to determine the size and shape of the sensitive area and sensitive volume of bacteria, viruses, and enzymes.^(7,19,36,38,49,50,51,55) In cells and enzymes, the sensitive volume can be different for different functions of the same enzyme, or cells. The characteristics of a physiologically active molecule such as an enzyme depends upon the location of the active sites and the spatial arrangement of the other constituents of the enzyme which determine which substrates can be acted upon by the active sites.

Investigations carried out with ionizing particles, whether concerning the inactivation of viruses,^(37,62) the killing of bacteria,^(36,40,66) or the inactivation of enzymes^(7,14,48,49,51,55) prove that the mean lethal dose increases with ion density, the survival curves are exponential, and the effects noted are independent of intensity⁽³⁹⁾ and of the temperature⁽⁵²⁾.

With biological systems in which the chromosomes are of such a size as to be observable under a microscope and ultraviolet light, x-rays, alpha particles, or protons are used, it has been shown^(10,60,66,67,68) that radiations cause more observable effects when they interact with chromosomes than with the cytoplasm. A dose of 28,300 protons delivered to the cytoplasm failed to cause any observable effects, but a dose of only 20 protons delivered to the chromosomal area caused an observable effect.

Lea⁽³⁸⁾ reported a value of 4×10^3 roentgens for the mean lethal dose for the irradiation of Escherichia coli with beta rays and a value of 24×10^3 roentgens with alpha rays illustrating that as the ion density increases, the mean lethal dose also increases.

Experimental work performed on Drosophila melanogaster^(1,15) has given data which when plotted results in curves which would be expected when multiple chromosomal breakage is produced from a single proton hit. The survival or inactivation curves were exponential.

When biological material is bombarded with fast neutrons, 92.5% of the damage is due to so called knock on collisions with protons. The fast neutrons are scattered by hydrogen nuclei which may receive up to 100% of the total energy of the neutrons which scatter them. These protons

are now heavily ionizing particles traveling in relatively straight paths and their specific ionization is so high that the probability of their causing one ionization in traveling through a sensitive volume is unity. The probability of a hit by a fast neutron thus depends on the target area. One could hope to obtain a cross section of the sensitive volume of a bacteria or virus from fast neutron bombardment experiments. Some few experiments have been performed on biological specimens in solution.

A value for the mean lethal dose for fast neutron irradiation of Drosophila was obtained and found to be approximately 1.8×10^{11} fast neutrons per cm^2 .

Escherichia coli exposed to a beam of fast neutrons while in broth had a mean lethal dose value of 7.1×10^3 v units* or 7.1×10^3 rep.⁽⁴⁰⁾ In the energy range used in that experiment, 7.1×10^3 rep. is equivalent to 1.4×10^{12} (24) fast neutrons per cm^2 .

Escherichia coli was plated on agar and exposed to a beam of fast neutrons⁽⁵⁶⁾ from the $\text{Be}^9(d,n)\text{B}^{10}$ reaction and the mean lethal dose was determined to be approximately 2×10^3 n units.** Therefore the mean lethal dose was 5×10^3 rep. Other experiments produced values of the same

* one v unit = 1 rep.⁽⁴⁰⁾

** one n unit = 2.5 rep.⁽²⁴⁾

order of magnitude⁽³⁰⁾. Thus, it appears that the mean lethal dose for fast neutron irradiation of Escherichia coli is of the order of 10^{12} fast neutrons per cm^2 .

Since the curve showing the relationship between survival and dose was exponential for all these cases, one may assume that any indirect effects were of negligible consequences. However, all the experiments performed up to the present have been with solutions which could have contained small amounts of radiation poisons after irradiation. Thus, an experiment was planned with lyophilized bacteria to see if the mean lethal dose would be of the same order of magnitude. The present experiments were performed to determine the effective cross section of the lyophilized bacterium, Escherichia coli.

THEORY

Indirect Effects.

When solutions are exposed to ionizing radiation, changes occur in the chemical structure. This is noted in mediums containing biological substances.

In most biological systems up to 80% of the constituents are water. When a water molecule is ionized, an electron is ejected leaving behind a positively ionized water molecule. The positive ion dissociates into a hydrogen ion and a free hydroxyl radical. The ejected electron travels in the water a distance, determined by its energy, and is then captured by another water molecule which in turn dissociates into a hydroxyl ion and a free hydrogen atom.

The free ions and the highly reactive free radicals which are formed will attack chromosomes and active sites on enzymes. This so called indirect effect spreads the radiation over a wider area to give experimental values for cross sections which are larger than those obtained when this action is not present. The distance a free radical will travel before interacting with a sensitive portion of the target is variable. This makes the determination of sensitive areas uncertain. Target size determinations have meaning only when the action is predominantly direct.

Accurate determinations can be made theoretically only on dry viruses, enzymes, hormones, and lyophilized micro-organisms.

Target Theory.

The target theory developed by Lea⁽⁴⁸⁾ is based on the assumption that the primary act of radiation absorption takes place in a very localized volume, and that the observed biological effect is due to a single ionization.

For the assumptions to be valid for a given radiation, the following criteria must be met:

1. The survival curve must be exponential.
2. The effect of a given dose must be independent of the temperature, the intensity at which it is given, and the manner in which it is fractionated.
3. The mean lethal dose must increase with ion density.

Experiments referred to in this report, have shown that these three criteria are met for many biological effects from ionizing radiation, and particularly for dry or lyophilized viruses, enzymes, hormones, and should be true for lyophilized bacteria.

In accord with the first criterion given above, a semilog plot of survival vs. dose results in a straight line if the action is direct. This curve represents the equation:

$$n = n_0 e^{-bd}$$

[2]

where n is the number of survivors, n_0 is the original number of specimens to be irradiated, d is the dose given to the sample and b is the probability that some specific absorption event occurs per unit dose in the vital volume or area. The mean lethal dose which is the dose which will kill all but 62.8% can be read from the curve itself.

If instead of a straight line, a curve is obtained which shows no effect at low values of dose and a sigmoid curve with increasing dose, the idea of a single hit or inactivation dose is no longer valid.

If the probability of occurrence of an event in a single trial is p , the chance that it will occur exactly s times in n independent trials is:

$$P(s) = \frac{n!}{s! (n-s)!} p^s (1-p)^{n-s} \quad [3]$$

For small numbers the above can be approximated by:

$$P(s) = \frac{(np)^s e^{-np}}{s!} \quad [4]$$

If the expected average number of hits in each trial is $\mathcal{E} = np$, then

$$P(s) = \frac{\mathcal{E}^s e^{-\mathcal{E}}}{s!} \quad [5]$$

$P(s)$ is the probability of exactly s hits in a path length where s is the actual number of hits. If a single ionizing event is effective, multiple hits must also be effective,

thus complicating the determination of $P(s)$. But if the probability of nonoccurrence is calculated and subtracted from unity, i.e. when $s = 0$:

$$P(0) = e^{-\epsilon} \text{ (i.e. probability of no hit, survival) } \quad [6]$$

Bombardment in Area.

A consideration of the amount of energy deposited per 100 Å by protons, deuterons, and alphas and the amount of energy released per ionization will attest to the high specific ionization occurring along the path of these ionizing particles.

If $P(0)$ is the probability of survival, N equals the number of survivors, and N_0 , the original number of specimens:

$$N = N_0 P(0) \quad [7]$$

but $P(0) = e^{-np}$ where n is the number of trials which in this experiment is equivalent to the number of attempts at hitting a sensitive area, i.e. the total dose (D) delivered and p is the probability of occurrence of an event, i.e. the cross section A . The following equation leads to a determination of the sensitive area:

$$N = N_0 e^{-AD} \quad [8]$$

When $N = .37N_0$

$$\ln N/N_0 = -1 \quad \text{and} \quad A = -1/D$$

D can be expressed in units of particles per cm^2 and thus A will have units of area and can be thought of in terms of a sensitive area.

Neutrons.

From a knowledge of the strength of a neutron source, the value for the number of neutrons per cm^2 at any distance from the source can be determined. The energy deposited in a sample at a point can be determined by means of the following equation. The energy deposited per unit mass (E_m) may be represented by⁽²⁵⁾:

$$E_m = kE \sum_{i=1}^n n_i \sigma_i \frac{2 A_i}{(1+A_i)^2} \quad [9]$$

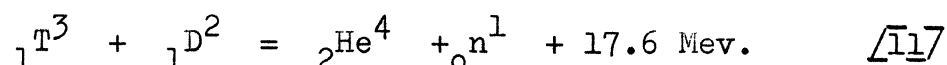
Where E is the energy of the impinging neutrons, k is the number of neutrons per cm^2 , n_i is the number of atoms of type i per unit mass of the medium, σ_i is the cross section for the ith type of atom for the neutron energy used, A_i is the ratio of the ith particle to the mass of the neutron, and \sum_i represents the summation over all ith atoms.

Since 92.5% of the energy absorbed in tissue is due to the scattering of hydrogen atoms by neutrons; and if E is expressed in electron volts, the above equation can be approximated by

$$E_m = \frac{.54 k E n_H \sigma_H}{5.23 \times 10^{13}} \quad \text{rep.} \quad [10]$$

The approximate dose received by tissue exposed to a neutron flux of 10^9 neutrons per cm^2 as a function of neutron energy is given by Gray⁽²⁵⁾. The value for 10^9 neutrons of energy 14 Mev is approximately 5 rep. These values were used to obtain a correlation between rep values obtained by other authors and neutron fluxes and to determine the range of flux necessary for a mean lethal dose.

To obtain a sufficient number of neutrons, the $\text{T}^3(\text{d},\text{n})\text{He}^4$ reaction was used:



The neutrons were obtained by bombarding a tritium target with deuterium ions. The tritium target prepared by the Isotopes Division, Oak Ridge National Laboratory, consisted of tritium absorbed on zirconium deposited on platinum. The cross section for this reaction as a function of bombarding energies has been determined theoretically^(13,17) and experimentally^(3,26) and found to have a maximum of approximately 4 barns at a bombarding energy in the neighborhood of 125 kev.

Angular distribution measurements⁽⁴⁾ indicate spherical symmetry in the center of mass system, and experiments performed show that at low bombarding energies there is at most an asymmetry of only a few per cent in the lab system.

The energy of neutrons at any given angle is almost monochromatic. There is at most a 100 kev spread of energies for thick targets⁽²⁶⁾. Nomographs prepared by McKibben⁽⁴⁴⁾ show a range of energies 13.4 Mev. through 14.25 Mev. for an angular distribution of 135° to 45° with an average energy at 90° of 13.85 Mev. Thus the distribution of energies as well as the distribution of neutrons is practically isotropic in the laboratory system.

In various mediums, a neutron colliding with a proton can lose up to 100% of its energy through a scattering collision. On the average, it loses $1/eth$ of its energy in each collision. Though scattering in the center of mass system is spherically symmetric; in the laboratory system, it is a function of the scattering angle, expressed as $\cos \theta = 2/3A_1$, where θ is the scattering angle and A_1 is the ratio of the masses of the two particles. For the case of scattering of a neutron by a proton, $A_1 = 1$ and $\cos \theta = 2/3$, and the scattering is preferentially in the forward direction in the laboratory system.^(21,42) Thus, the neutron through an elastic scattering process, produces a heavily ionizing particle with an energy of approximately 9 Mev. The specific ionization of such a particle is so high that the probability of ionizing a molecule in the sensitive volume is unity and the value obtained in this experiment is for a sensitive area.

METHODS

Sample Preparations.

A wild strain of Escherichia coli was used in this experiment.

Cultures were maintained on agar slants and transferred to the nutrient broth solutions for incubation at 37° C. After twenty hours, 0.1 ml. portions of broth culture were pipetted with special long neck pipettes into previously prepared cotton stoppered and sterilized lyophilization tubes. The cotton was replaced in these tubes and the tubes placed in a consecutively numbered rack. The tubes were then placed in the holders of a lyophilizer and the contents were freeze dried at 30 microns of Hg. When the tubes had returned to room temperature, i.e. all the free water had sublimed, the tubes were sealed while under pressure, removed, and placed in their original positions in the tube rack. Thirty-six tubes were freshly prepared for each run.

The samples to be exposed were placed in a special sample holder (figures 2 and 6), arranged in a circular pattern and the position of each tube noted. The tubes were carefully positioned in a circle about the target. In all experiments, the even numbered tubes were exposed and the odd numbered tubes retained for controls. Except for the short period of time when the even numbered tubes were being

irradiated, all of the tubes were retained in the tube rack in order that all would be subjected to the same effects.

Radiation.

Using portable neutron and gamma detectors, radiation levels were checked in the building each time an irradiation was made.

The secondary target containing the vicor observation window (figure 5) was placed on the target flange of the Cockcroft-Walton accelerator and the beam visually focused. After the beam had been centered on the vicor window by leveling and positioning screws, the secondary target was removed and the tritium target placed on the accelerator (figure 7). The beam current and focus were then checked by means of a probe which is built into the system and the beam was next allowed to fall on the tritium target itself.

In this experiment, to average errors which might arise because of anisotropy, those sample tubes in the outer positions were changed spatially, so they would obtain equal energies and equal numbers of neutrons. With a bombarding energy of 150 kev., the deuteron bombarded tritium target should give off approximately 10^8 neutrons per microcoulomb of deuterons. (26)

When BF_3 neutron detectors indicated sufficient irradiation, the Cockcroft-Walton accelerator was turned off,

and the sample holder reversed. After a similar irradiation period with the samples in the new position, the machine was turned off and the samples were removed. The number of counts recorded by the scalers for each position was recorded.

After irradiation, each of the control tubes and the irradiated tubes were processed as follows:

Plating Techniques.

The sample tubes were placed in a 70% alcohol solution and a pair of tweezers, a pair of pliers, and a 1/4" steel rod were placed in another 70% alcohol solution for ten minutes. The tubes were removed from the alcohol solution by means of the tweezers and pliers, the tubes being held by the pliers until the alcohol evaporated. Then the tubes were placed in a 99 ml. dilution blank which was agitated until the tube broke. The dilution bottle top was removed and the steel rod used to crush the tube; the top replaced and after the bottle was thoroughly agitated to obtain an even distribution of the contents, blanks containing the bacteria were placed in a transfer hood.

At the end of ten minutes, the dilution blank was again agitated and a one ml. portion transferred to a 99 ml. dilution blank was thoroughly agitated and placed to one side in the hood. One ml. and 0.1 ml. portions were transferred from the first dilution blank to three petri dishes each.

Nutrient agar was added to the six dishes and they were thoroughly swirled in order to obtain an even distribution of the bacteria.

When ten minutes had elapsed after inoculation of the second dilution blank, it was again thoroughly agitated; and the same procedure followed as for the first dilution blank.

Dishes were incubated at 37° C. for 24 hours. Only those plates containing between 30 and 300 colonies were accepted as countable

Determination of Source Strength.

The flux ϕ at a distance r from a point neutron source of strength S is represented by the inverse square relationship:

$$\phi = \frac{S}{4\pi r^2} \quad [127]$$

In the present experiment, the source was so small and the distance from the detector to the source so great, that the source was treated as a point source in determining its strength. With a long counter as a detector, the flux is given by:

$$\phi = \frac{CR}{\epsilon A_d}$$

where CR is the observed counting rate (table 2), A_d is the area of the face of the long counter and ϵ is the efficiency

of the counter. Thus if the CR is known, the source strength can be obtained from the equation:

$$S = \frac{CR \ 4 \ \pi r^2}{\epsilon A_d} \quad [13]$$

For this experiment, the area of the counter was 81.07 cm² and the distance from the source to the counter was 500 cm. The long counter used in this experiment was calibrated to have an efficiency of 10⁻³ neutrons per count when used with the ${}^3_1\text{T}(\text{d},\text{n}){}^4_2\text{He}$ reaction. Thus the source strength was related to the CR through the equation:

$$S = 9.69 \times 10^6 \text{ CR neutrons/sec.} \quad [14]$$

Determination of Flux.

Because of the finite size of the target, the shape of the samples, and their arrangement, a numerical integration was necessary to determine the flux for each sample. The area of the target source was ascertained and divided into 16 equal parts. The integration was carried out twice, once for the outer samples and once for those samples whose center points lay on the same plane as the target.

The sample tubes were cylindrical in shape with hemispherical ends. A measurement was made of the thickness of the tube and from large scale drawings of the tubes, correct dimensions for the inner sample coated portions of the

cylinder and hemisphere were obtained. The cylindrical portion of the sample tube was divided into eight equal areas and the hemispherical portion was divided into 24 equal areas. The areas of these parts were determined and the total areas of these portions calculated (table 1).

A coordinate system was set up and the center of each target section and of each sample area was determined. The equations for the cylinder and the hemisphere were obtained and the gradient of each was taken. From these, the value of the normal to the surface at each center point was calculated and the angle between a line from the center point of each target area to the center point of each sample area and this normal line was obtained. Also, the distance from each target area section to each sample area was found and the summation of the $\cos \phi/r^2$ values from each target area to each sample area then determined (table 1).

The integrated source value for each run was obtained by means of the long counter as previously described. All the values obtained as listed above were applied to obtain the flux at the location of the sample for the final determination of the sensitive area.

Flux Determination at the Location of the Samples.

The solid angle subtended at a point distance r , is given by

$$d\omega = dA_s \cos \phi / r^2$$

Table 1

Target area, area of samples, and $\cos \phi/r^2$ values for the determination of flux at sample position.

<u>Area Values</u>	<u>dA_s</u>	<u>Total Area</u>
Hemispheres (sample tubes)	0.0355 cm ²	0.852 cm ²
Cylindrical (sample tubes)	0.0456 cm ²	0.365 cm ²
Disc (Target)	0.0198 cm ²	0.317 cm ²

$\cos \phi/r^2$ values		
<u>Sample Position</u>	<u>Cylindrical Portion</u>	<u>Hemispherical Portion</u>
Outermost Position	38.0 cm ⁻²	134. cm ⁻²
On Axis of Target	52.8 cm ⁻²	281. cm ⁻²

If the source strength per unit area is dS/da , then the total source strength is $S = \int dS/da (da)$ and if this source strength is uniform over the entire area, $S = \int S/a da$. If the average flux per unit area is $\bar{\phi} = d\phi / dA_s$, then the

total flux is $\bar{\phi}A_s = \int d\phi / dA_s (dA_s)$ or

$$\bar{\phi} = 1/A_s \int_{A_s} d\phi / dA_s (dA_s) = 1/A_s \int_{A_s} d\phi \quad \text{but}$$

$$d\phi = \frac{S d\omega}{4\pi r^2}, \quad \text{so} \quad d\phi = \frac{S dA_s \cos \theta}{4\pi r^2} \quad \text{and}$$

$$\bar{\phi} = \frac{S}{4\pi A_s a} \int_{A_s} \int_a \frac{\cos \theta da dA_s}{r^2} \quad [15]$$

The average flux values are tabulated in Table 4.

Determination of the Sensitive Area.

Making use of the equation [8], $N = N_0 e^{-AD}$, where N_0 is the number of original bacteria before irradiation (or number of bacteria in control tubes), N is the number of bacteria surviving irradiation, D is the dose in neutrons per cm^2 (i.e. ϕ in equation [15]), and A is the sensitive area.

Since preliminary experiments had shown a large variation between tubes due to lyophilization alone, the experiment was rearranged so a large number of tubes could be exposed to an equal number of neutrons. These tubes were compared to a large number of unirradiated control tubes to compensate for tube variation.

In each run, twelve tubes received equal amounts of radiation and a second set of six received equal amounts of radiation. Eighteen tubes were held for unirradiated controls. An average for all eighteen control tubes was obtained and average values for the sets of twelve and six obtained. These values were subjected to the analysis which follows.

Determination of the Area.

If the ln of the ratio of viable counts of organisms surviving in the irradiated tubes to viable counts in the control tubes was said to be the experimentally determined value and the product of the area and the dose said to be the calculated value, then using the following reasoning, the value for the sensitive area A can be determined.

For a given value of x_1 , we have an observed value of y (designated as y_1) and a calculated value of y (designated as y_1') obtained by substituting x_1 in the equation

$$y_1' = \alpha + \beta x_1 .$$

If this equation for the line fits the data, then to satisfy the criteria of least squares, the constants α and β must

be such that $\sum_{i=1}^n (y_i - y_i')^2$ is as small as possible. Thus

by taking the differential of $\sum_{i=1}^n (y_i - y_i')^2$ with respect

to y_1' and setting this differential equal to zero, the value of y_1' that makes this a minimum can be determined.

In the present experiment, y_1 , is the experimental value ($\ln N/N_0$) and y_1' equals the calculated value (AD) and

thus $\sum_{i=1}^n (y_1 - y_1')^2 = \sum_{i=1}^n (\ln N_1 - \ln N_{01} + AD_1)^2$. Taking

the differential with respect to A and setting it equal to zero,

$$(\ln N_1 - \ln N_{01} + AD_1)D_1 = 0, \text{ or}$$

$$A = \sum_{i=1}^n \frac{(\ln N_{01} - \ln N_1)D_1}{D_1^2} \quad [16]$$

MATERIALS

Cockcroft-Walton Accelerator.

The Cockcroft-Walton⁽⁴³⁾ accelerator used in this experiment was constructed at the Virginia Polytechnic Institute by graduate students under the direction of Dr. A. Robeson. The high voltage supply is rated at 250 kv., base pressure is 10^{-6} mm. Hg., maximum current obtainable 120 microamperes of beam current. Deuterium is fed into the radio frequency excited ion source via a pallidium leak. For this experiment the high voltage was maintained at 125 kv. and the beam current varied from 100 microamperes to 5 microamperes.

Long Counter.⁽⁴¹⁾

A 1/2" diameter BF_3 counter is surrounded by a layer of paraffin eight inches in diameter which in turn is surrounded by a 1/2" layer of B_2O_3 (anhydrous boric acid) and an additional three inch layer of paraffin. The entire arrangement is enclosed in a sheet metal shell 40 mils thick. The inner paraffin layer and counter are recessed $2\frac{1}{2}$ " from the front face of the outer layers. The entire counter arrangement is $16\frac{1}{2}$ " overall in length and 15" in diameter. It is supported on a steel table and a screw attachment makes possible the positioning of the counter so it can be aimed at a source which is not on its own level. This counter was placed 500 cm. from the neutron source.

BF₃ Detectors.

Diameter: 1/2"
 Operating voltage: 1500 volts
 Pressure: 40 cm. of Hg.
 Active length: 10.5"
 Manufacturer: N. Wood Counter Laboratories

Supplemental Counter.

A 1/2" BF₃ counter was embedded in thick slabs of paraffin and positioned one meter from the neutron source. It was used to monitor counter number one in case of a malfunction in the primary counter.

Associated Electronics.

Pre amplifier: #219A, manufactured by Atomic Instrument Company.

Linear amplifier: #218, manufactured by Atomic Instrument Company.

Ultra scaler: #192A, manufactured by Nuclear Chicago.

Flanges Holding Target (Figures 3 and 4).

Two brass flanges were constructed to be bolted on to the Cockcraft-Walton accelerator to hold the target assembly and to allow for a flow of cooling water between the two flanges. The flange holding the target was recessed so the sample holder assembly was supported by the sides of the recess.

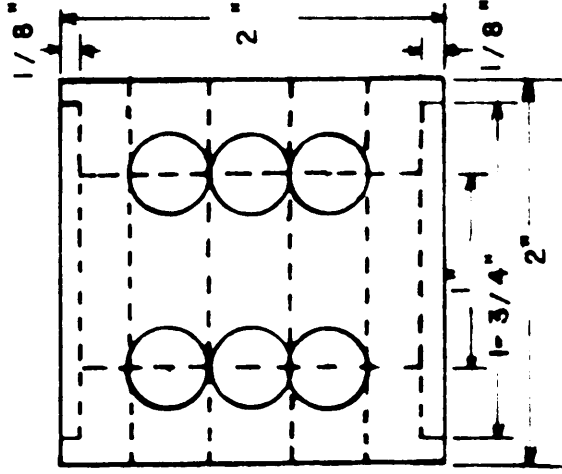
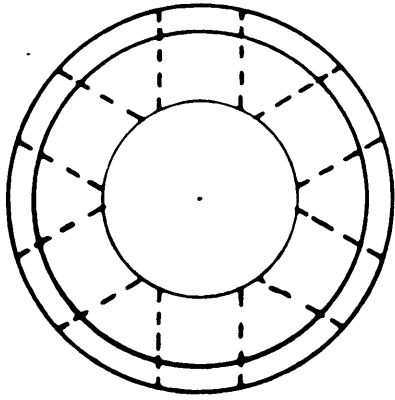


FIG. 2 SAMPLE HOLDER

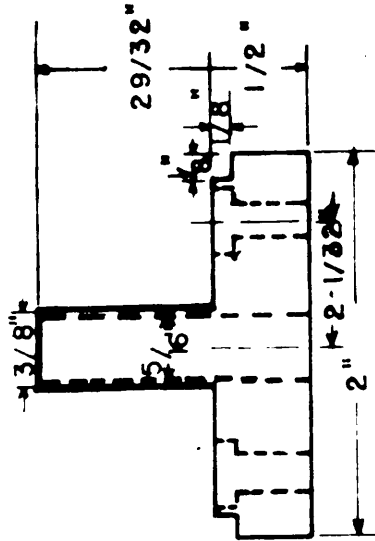
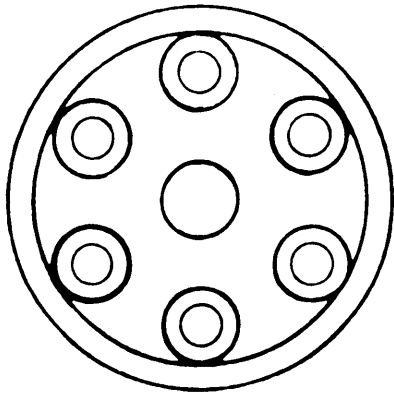


FIG. 1 TARGET ASSEMBLY

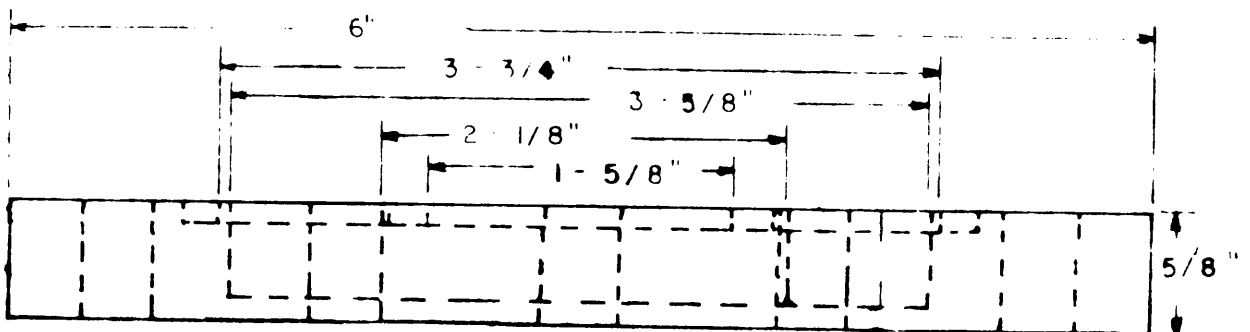
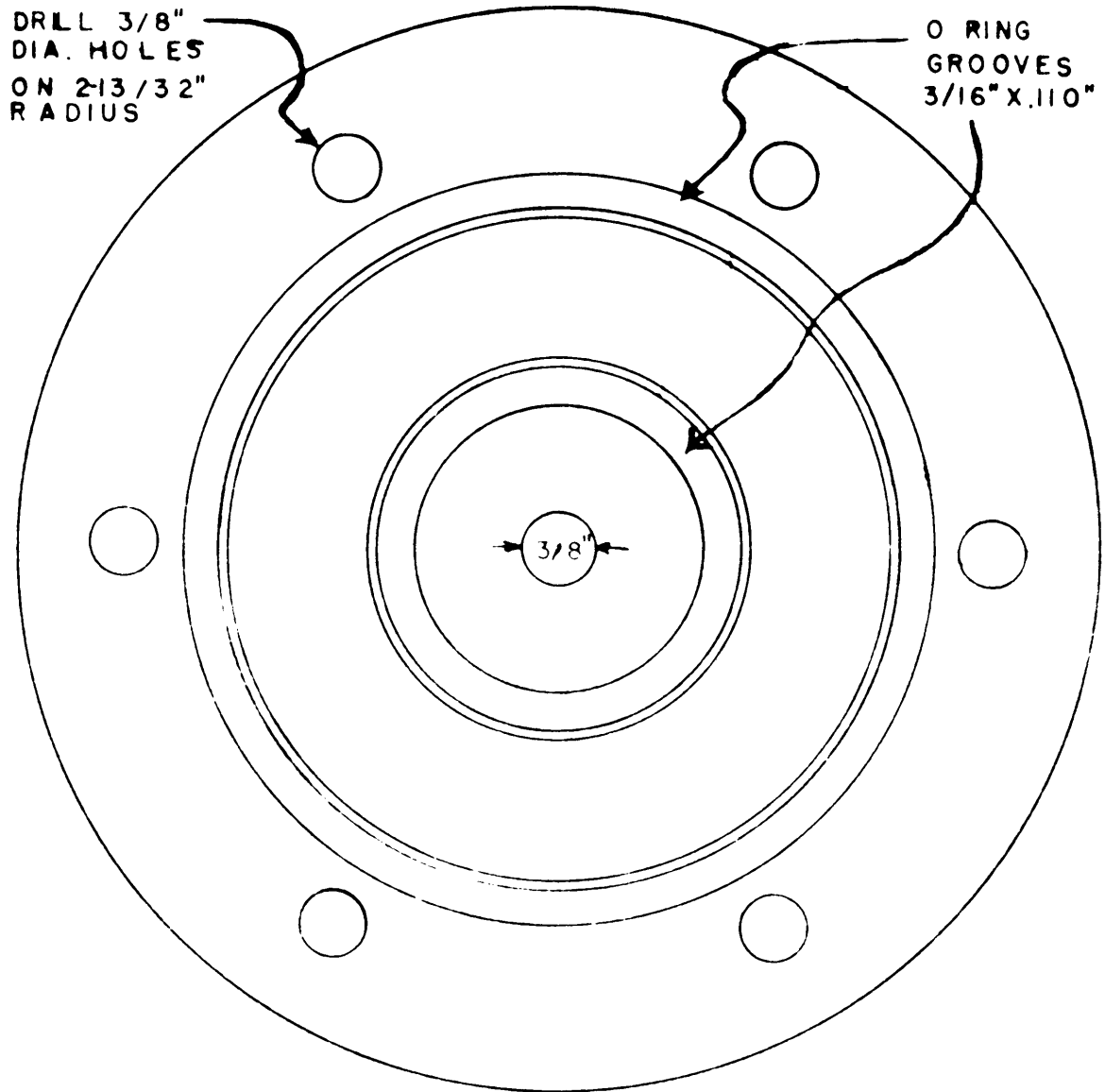


FIG. 3 COOLING FLANGE

Tritium Target Holder (Figures 1 and 6).

This assembly was constructed from one piece of brass. The target end consisted of a tubular portion $3/8$ inch outside diameter and $5/16$ inch inside diameter. A $5/16$ inch circle was cut from a tritium target by means of a specially made punch and die and placed in the end of the target assembly. A short piece of $5/16$ o.d. copper tubing was placed in the tubular end to press the target firmly against the end of the holder. This left a $1/4$ inch diameter target exposed to the deuterium beam.

The flange end of the assembly was constructed to fit in the recess of the holder flange and also contained a shoulder so the sample holder could fit between the target holder and the holder flange. Holes were drilled and counter-bored to hold tightening screws.

Secondary Aligning Target (Figure 5).

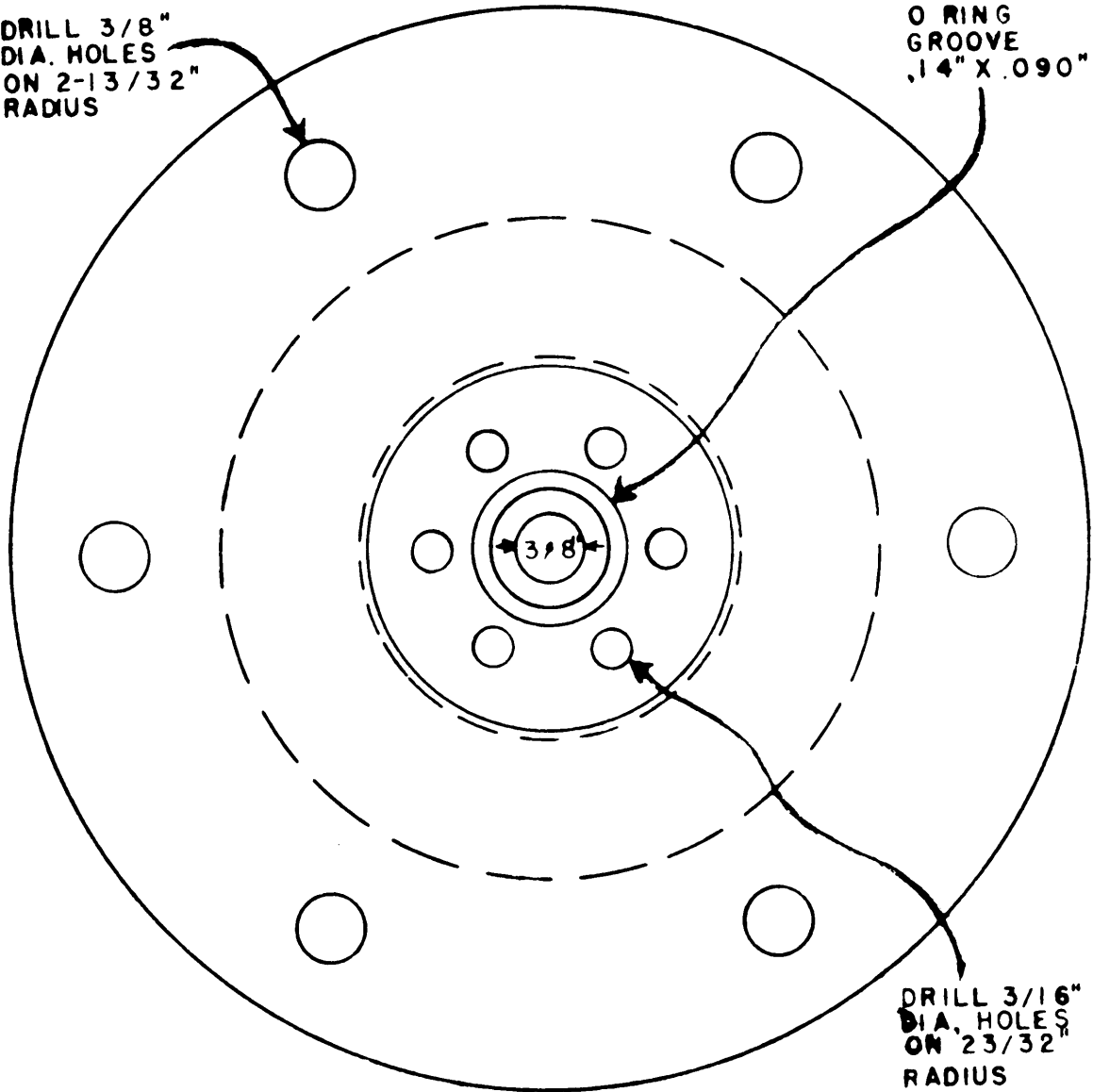
The design of this holder is similar to the tritium target holder. The end was cut off the tubular end and a vicor window affixed to it by means of sealing wax.

Target Positioning Device.

Two short pieces of $1/4$ " x 1" angle iron were cut and bent to make upright posts. They were welded to a $1/4$ " steel plate at a distance of eight inches apart. Holes were drilled and tapped into the tops of the angle iron so $3/8$ " steel bolts

DRILL 3/8"
DIA. HOLES
ON 2-13/32"
RADIUS

O RING
GROOVE
.14" X .090"



DRILL 3/16"
DIA. HOLES
ON 23/32"
RADIUS

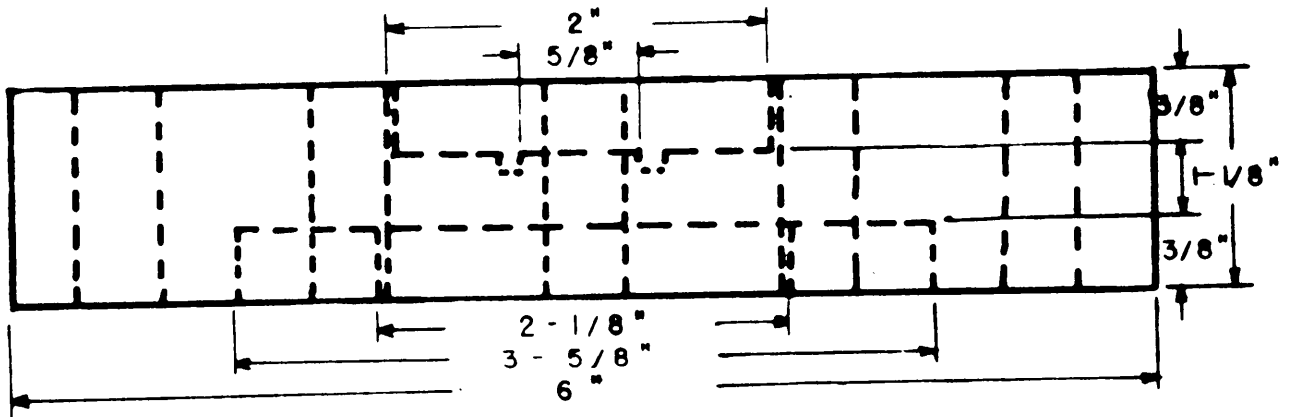


FIG.4 TARGET HOLDER FLANGE

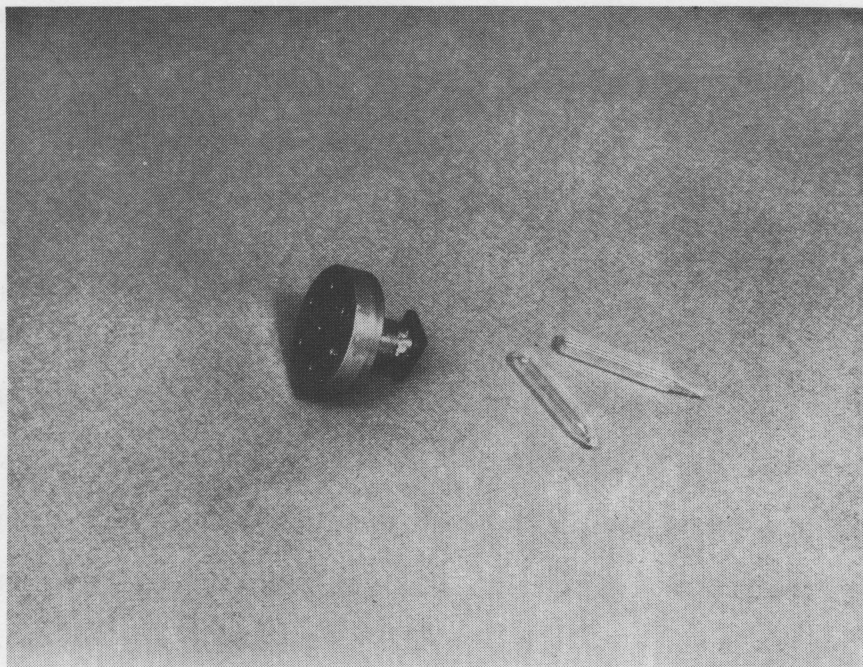


FIG. 5 SAMPLE TUBES
AND ALIGNING TARGET

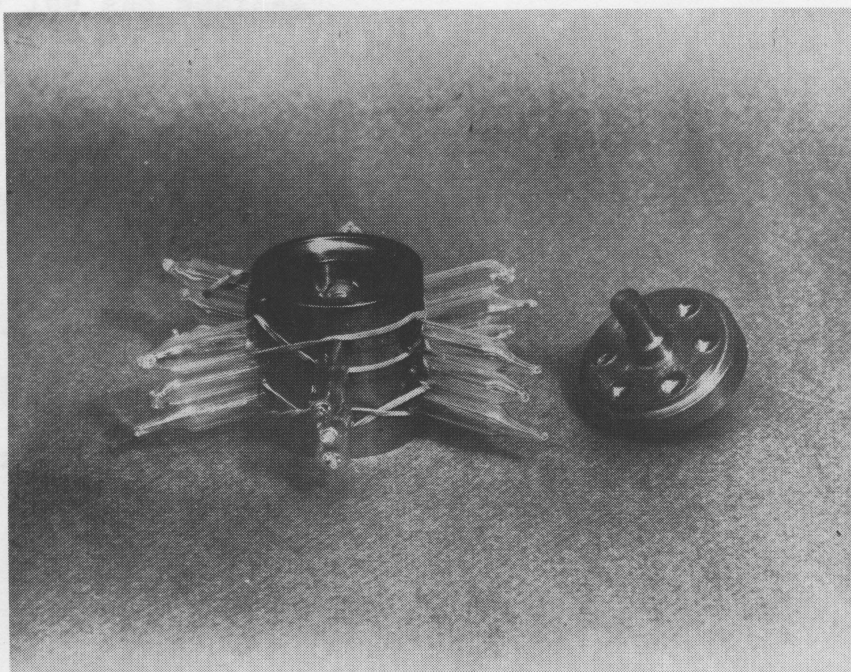


FIG. 6 SAMPLE HOLDER,
TUBES, AND TARGET

could be run back and forth against the sides of the flanges in order to position the target laterally. The steel plate was drilled and tapped at three locations to take 3/8" steel bolts. These were used to position the target vertically.

Sample Holder (Figures 2, 6, and 8).

A two inch brass rod, two inches long, was drilled out so it had a one inch diameter hole. Recesses were cut into each end to fit the holder flanges. Three sets of six radial holes 1 cm. in diameter and 60° apart were drilled through the sides. They were positioned so the center six were in the exact center of the rod and the other two sets were equidistant from the ends.

Lyophilizer.

Capacity: 38 tubes

Vacuum: 30 microns when ice trapped

with dry ice and acetone.

Lyophilizing Tubes.

Pyrex #9820 10 x 75 mm.

These tubes were heated approximately 2 cm. from the open end and drawn out to a capillary at this point. The capillary size was large enough to allow a drawn out capillary of a 1 ml. pipette to pass to the bottom of the lyophilizing tube.

Phosphate Buffer.

Thirty-four grams of KH_2PO_4 were dissolved in 500 ml. of distilled water; 175 ml. of 1 N NaOH was added to the solution and the total volume was made up to 1 liter with distilled water. 100 ml. portions of this solution were placed in dilution blank bottles and glass beads were added to one half of these bottles. All were sterilized in an autoclave for 15 minutes at 121° C. at 15 pounds pressure. (Standard methods for the Examination of Dairy Products 10th edition (1953) American Public Health Association, Washington, D. C.)

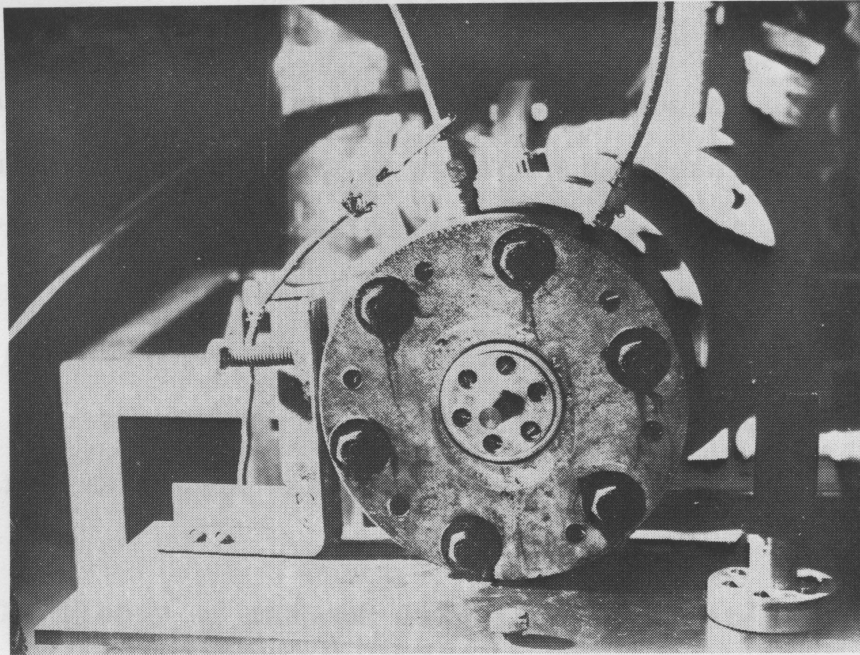


FIG. 7 TARGET MOUNTED
ON COCKCROFT WALTON

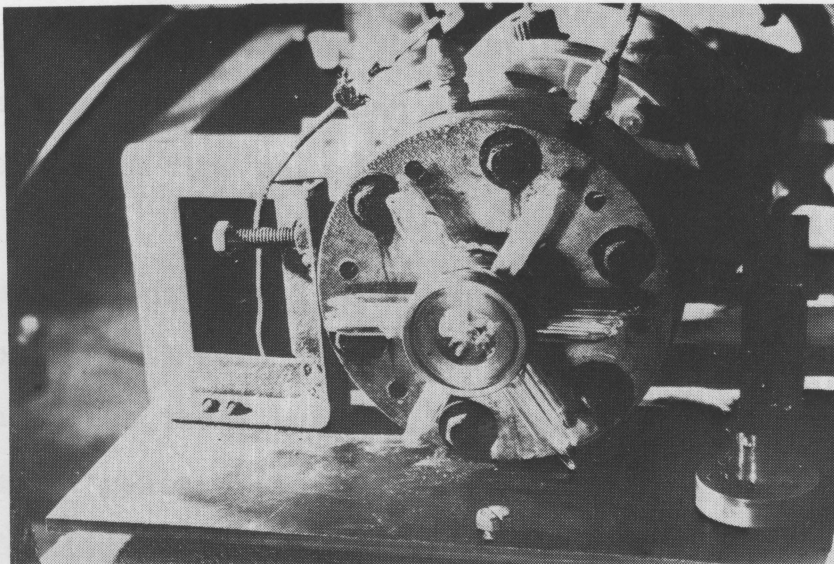


FIG. 8 SAMPLES MOUNTED
IN EXPOSURE POSITION

RESULTS AND DISCUSSION

An analysis of variance was performed on all data to determine if there was a significant difference between the numbers of viable cells in the irradiated and control groups. Only one run showed a statistically significant difference. It is evident that the tremendous variation between tubes within treatments obscures the difference between treatments. The large variation was caused by the lyophilization process. (Table 6)

The theory of maximum likelihood estimates was used to determine the sensitive area and the standard deviation. These values are shown in Table 5.

Under the conditions of this experiment, the F values could be made larger and the standard deviations smaller only by using more samples at each irradiation position. This would require placing the samples at a much greater distance from the target than used here and would increase the irradiation time many fold to obtain the same dose per tube.

There are now available more efficient lyophilizers which do not kill such large fractions of the original bacteria. Also there are available special squirt type guns used in genetic studies on viruses which would increase the uniformity of contents in the original tubes. New ion

sources available for the Cockcroft-Walton accelerator lead to a thousand fold increase in the rate of fast neutron production. These high fluxes would allow an experimenter to use many more tubes in each experiment thus decreasing the effect of variability due to lyophilization.

This experiment demonstrates the difficulties which arise in working with biological systems. Due to their inherent variability one must work with large sample sizes in order to overcome or average out the variables which are not germane to the question under consideration. The value of statistical techniques in this field is evident.

It must be noted that the aforementioned apparatus or statistical analysis would not remove all of the uncertainty of the sensitive area which must remain regardless of the refinement of the apparatus. This sensitive area may vary from bacterium to bacterium so an average value with certain minimum and maximum limits is all one can hope to ascertain.

This experiment demonstrated that the sensitive area due to direct action was of the same order of magnitude as that due to indirect action^(37,56) and represents approximately 0.01% of the total area.

Table 2

Fast neutron flux recorded at long counter position.

(Counts Recorded)

<u>Run</u>	<u>Position 1</u>	<u>Position 2</u>	<u>Total</u>	<u>Time of Exposure</u>
1	712,273	714,012	1,426,285	1 hr. 40 min.
2	550,196	536,800	1,086,996	1 hr. 47 min.
3	283,497	283,510	567,007	3 hrs. 20 min.

Distance from long counter to source 500 cm.

Table 3

Ln. of average colony count of bacteria surviving.

<u>Run</u>	<u>Control</u>	<u>Near Position</u>	<u>Outer Position</u>
1	4157.30 x 10 ³	614.80 x 10 ³	1731.40 x 10 ³
2	5915.20 x 10 ⁴	1527.00 x 10 ⁴	3695.10 x 10 ⁴
3	3122.90 x 10 ⁵	770.70 x 10 ⁵	1773.20 x 10 ⁵

Table 4

Neutron flux at sample position.

(In neutrons per cm²)

<u>Run</u>	<u>Near Position</u>	<u>Outer Position</u>
1	686 x 10 ⁹	366 x 10 ⁹
2	523 x 10 ⁹	279 x 10 ⁹
3	273 x 10 ⁹	146 x 10 ⁹

Table 5

Sensitive area of bacteria.

<u>Run</u>	
1	4.7 ± 1.8 x 10 ⁻¹² cm ²
2	3.2 ± 4.3 x 10 ⁻¹² cm ²
3	2.9 ± 4.0 x 10 ⁻¹² cm ²

Table 6

Analysis of variance between exposed and unexposed bacteria.

<u>Source</u>	<u>Sum Squares</u>	<u>Degrees of Freedom</u>	<u>Mean Squares</u>	<u>F</u>
<u>Run 1</u>				
Between Treatments	827.566	2	413.783	1.54
Within Treatments	7537.650	28	269.202	
Within Tubes	114.335	63	1.815	
F 25 (2,28) = 1.46				
F 10 (2,28) = 2.50				
<u>Run 2</u>				
Between Treatments	602.01	2	301.01	1.008
Within Treatments	8960.527	30	298.68	
Within Tubes	212.96	67	3.03	
F 25 (2,30) = 1.45				
<u>Run 3</u>				
Between Treatments	460.6258	2	230.3129	3.072
Within Treatments	2399.0221	32	74.97	
Within Tubes	131.922	71	1.86	
F 10 (2,32) = 2.48				
F 5 (2,32) = 3.32				

SUMMARY

Experiments were performed to determine the sensitive cross section of a lyophilized bacterium, Escherichia coli, to fast (14Mev.) neutrons. The neutrons were obtained from the $H^3(d,n)He^4$ reaction and the source strength determined by means of a long counter. The total flux at the site of irradiation was determined by numerical integration and found to be in the neighborhood of 10^{12} neutrons/cm. Target theory and a least squares determination gave a value of approximately 10^{-12} cm² for the sensitive cross section. This value is in agreement with those obtained by others for this bacterium irradiated in aqueous solution and on agar plates.

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and other students of the graduate school who assisted in this capacity. Also, a word of appreciation is due _____ for the construction and calibration of the long counter.

A word of thanks is extended to the parents of the author for their support and encouragement.

Though it sounds so small in comparison, thanks to my (silently long suffering, patient) wife and children.

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ABSTRACT

An Experiment was performed to determine the sensitive area of a lyophilized bacterium, Escherichia coli, to fast (14Mev.) neutrons. The neutrons were obtained from the $H^3(d,n)He^4$ reaction produced in the Cockcroft-Walton accelerator at Virginia Polytechnic Institute. The reaction provides a neutron source which is almost monoenergetic, symmetrical, has a low level of associated gamma rays, and gives a large number of neutrons per unit time. To insure that the maximum number of neutrons per cm^2 per unit time pass through the samples, a special target holder, and sample holder were constructed to position the samples close to the target's center. Source strength was determined with a long counter. The flux at the site of irradiation was determined by numerical integration.

One half of the lyophilized culture tubes were held for controls, the others were irradiated. After the latter had been irradiated, all were opened aseptically, plated on agar, and colonies counted. Because of the extreme variation between colony counts of lyophilized samples, a weighted least squares analysis was performed to estimate the sensitive area.

The total flux to which the bacteria were subjected was approximately 10^{12} neutrons/ cm^2 and the sensitive area

was estimated to be 10^{-12} cm². This agrees with values obtained by others for this bacterium irradiated in aqueous solution and on agar plates.

Since the average size of Escherichia coli is .5 x 1.5 microns, the sensitive area is approximately 0.01% of the total cell area.