

PRELIMINARY DEVELOPMENT OF A BIOLOGICAL
DOSIMETER

By

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PREFACE

The results of experiments dealing with the effect of gamma radiation on the chromosomes of an aquatic fly larvae are reported in this study. The experiments were designed to determine if the chromosomes of Chironomus displayed a degree of consistency in their response to ionizing radiation which would allow their use as a quantitative biological indicator of radiation absorbed dose. Experiments were divided into two phases: a) the determination of the nature of the chromosome damage produced by gamma radiation on the larval chromosomes, b) the determination of increases in total gross aberrations as a function of post-irradiation time.

This research is the outgrowth of ideas expressed by Professor Louis Hemphill in the spring of 1963. At that time, Professor Hemphill expressed the need for a method of directly determining the immediate as well as eventual effects of ionizing radiation on biosystems.

In completing this phase of his academic work, the writer wishes to express his sincere appreciation to the following individuals and organizations:

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CHAPTER I

INTRODUCTION

Statement of the Problem

There are several physical methods for measuring ionizing radiation. At the present time the most practical methods utilize electronic measurement. These measurement methods, while directly related to the accepted definition of the roentgen, are often insufficient for biological purposes. The roentgen unit directly relates ionization to radiation and is specific for x - or gamma radiation. In a mixture of dissimilar radiations the absorbed radiation dose required to produce specific biological damage is usually different for each radiation component. The extent to which various radiations differ from one another in their ability to produce biological damage is a measure of their relative biological effectiveness, or RBE. The International Commission on Radiological Units and Measurements (ICRU) in 1959 defined the RBE to be the inverse ratio of the respective doses which are required to induce a particular effect. The standard of radiation selected by the ICRU is "that x - or gamma radiation having a linear energy transfer in water of 3 Kev per micron path length at a dose rate of 100 rad per

minute" (1). The unit selected by the ICRU to represent the exposure dose of photon radiation is the roentgen symbolized by an upper case R (2). One roentgen is defined as "an exposure dose of x - or gamma radiation such that the associated corpuscular emission per 0.001293 grams of dry air produces, in air, ions carrying one electrostatic unit of quantity of electricity of either sign." (1). The ICRU has defined the absorbed dose of any ionizing radiation as "the energy imparted to matter by ionizing particles per unit mass of irradiated absorbed dose is the rad and is equal to 100 ergs per gram of material irradiated."

RBE values for various types of ionizing radiation vary significantly from radiation to radiation, and in the case of particulate radiation are strongly energy dependent. Some typical RBE values listed in increasing order of effectiveness are:

Photons and electrons of either sign	1
Slow neutrons	2.5
Fast neutrons	10
Heavy particles	1-20

In most cases, RBE values are listed and accepted as integral values. The magnitude and nature of these values casts some doubt on their validity when compared to the abundance of physical constants which are non-integral.

The uncertainty involved in RBE values makes it difficult to relate physically measured radiation absorbed doses to immediate or eventual biological consequences. In addition,

ionizing radiation is insidious with regard to long term biological effects, therefore, a physicochemical measurement of radiation may or may not be informative as to the eventual hazards of a particular absorbed dose of a particular type of ionizing radiation.

One approach to this problem is to measure the biological absorbed dose directly. The obvious difficulties which accompany this procedure are the nonlinear responses of biosystems to radiation, particularly at low doses, as well as the usual uncertainty associated with biosystems.

It is well established that chromatin material displays a high degree of radiosensitivity. In view of the enhanced sensitivity of chromatin, a chromosomal system was employed as a biological detector of radiation absorbed dose. The purpose of this study is to determine the extent and nature of aberrations induced in the salivary gland chromosomes of Chironomus plumosus from irradiation with Cobalt-60 gamma radiation, and, to compare the frequency and types of these induced aberrations with those found in control populations of the same organisms.

Scope of the Investigation

The development of the relationship between radiation induced chromosome damage and quantity of radiation or radiation intensity requires consideration of several related variables. In the ionization reaction several events occur spontaneously which potentially contribute to the damage of

a conjugate organic system. Gamma radiation absorbed in an aqueous system may produce a series of unusual ionic species, a region of highly concentrated electrons or a small region or intense heat (thermal spike). Specific knowledge of the interaction of gamma radiation with aqueous systems has not been achieved; however, one basic relationship, linear energy transfer, has long served as a reliable guide for establishing the relationship between the gamma radiation energy imparted to a system and the yield of the system.

Consideration of the multiphase processes inherent to biological systems and the difficulties of quantitatively measuring biological response to physical stimulus promotes comprehensive consideration of a limited number of variables. The major emphasis in this study, nature of the chromosome damage produced by gamma radiation, prompted a preliminary study of the system before completing the experimental design. Results of the preliminary study together with the findings of other investigations in this field served to define the scope of the investigation. A resume of the type of chromosome damage observed in the preliminary study are listed below:

1. Incomplete synapsis
2. Chromosomal fragments
3. "Clumped sections" of chromosomes
4. Pycnotic chromosomal surfaces
5. Chromosomal band diffuseness.

Incomplete synapsis, as related to this study, are defined as a separation between complementary sections of the chromatid

halves of a chromosome. A chromosomal fragment describes a complete break and separation of a portion of a chromosome. Since salivary gland cells do not divide and the chromosomes of these cells undergo endomitosis, once a chromosomal fragment has been produced, it is permanent for the life of the cell in which it is produced. Clumped sections of chromosomes mean that several major band divisions of a chromosome have undergone incomplete synapsis and chromatid halves have inter-twined. This aberration is usually accompanied by pycnosis. Pycnotic chromosomal sections are characterized by their sticky surfaces, which cause a chromosome to adhere to its nuclear chromosomal neighbors. The bands of a chromosome described as diffuse are broken down into a series of oval or spherical bodies of heterochromatin. These bodies seem to be distributed in a matrix of euchromatin. The boundaries of an altered chromosome may or may not be broken down depending upon the severity of the diffuseness. The condition of diffuseness is usually limited to a few bands or, at most, to two or three major band divisions.

It is important to note that the aberrations described as pycnosis and diffuseness are qualitative and are subject to the subjective opinion of the operator with regard to their extent.

In view of the aberrations observed in the preliminary experiment it is possible to separate the scope of this study into the following three generally defined areas:

1. The development of partial cytological maps of the chromosomes of the experimental animal.
2. The determination of the types and frequency of chromosomal aberrations occurring in a control population of the experimental organism.
3. The determination of the types and frequency of chromosomal aberrations occurring in an experimental population of the organism exposed to known doses of Cobalt-60 radiation.

Background Literature

Literature references were consulted to aid in selection of the experimental organism and provide information about radiation induced chromosome damage.

Balbani (3) is generally credited with the discovery of polytene chromosomes while studying Chironomus in 1881. Alverdes (4,5) rediscovered these giant chromosomes while engaged in some independent investigation on Chironomus in 1912. W. Beermann (6-13) has done extensive work on European species of this organism and has developed partial cytological chromosome maps of several members of this genus including Chironomus tetans.

H. J. Muller (14), in the late 1920's, gave quantitative proof that x-rays increased the number of mutations in the fruit-fly, Drosophila melanogaster. It had been known since 1898 that x-rays were capable of inducing biological damage, however, Muller was the first to suggest quantitative means

of analyzing this type of damage.

Dan Nelson, et. al., (15) at Oak Ridge National Laboratory have reported several inherited chromosomal aberrations in populations of Chironomus tetans inhabiting the Clinch River. These organisms have received continuous doses of ionizing radiations in excess of 213 millirads per year since 1943 (15). In addition, Nelson suggests that the larvae stages of Chironomus might be used as a biological dosimeter. It should be noted that these studies were directed toward the analysis and study of inherited aberrations; whereas, the study presented here is concerned with immediate somatic effects.

CHAPTER II

METHODS AND MATERIALS

The Experimental Organism

A variety of biological systems have been studied with respect to damage; however, only the chromosomes offer the advantage of structural consistency from generation to generation. In the larval salivary glands of the members of the order Diptera are found a unique type of chromosome which is quite large and can easily be studied microscopically. These chromosomes are formed by repeated chromatid divisions unaccompanied by either daughter chromatid separations or cell divisions. This process is known as endomitosis and the resultant bundle of threads, or chromonemata, is a polytene chromosome. Polytene chromosomes undergo endomitosis and the salivary glands do not divide, therefore, if a chromosome is damaged, the damage is permanent. This unique characteristic suggests that these chromosomes might exhibit a cumulative response to damage.

Chromosomes appear as long threads with a multitude of transverse bands. The individual bands exhibit a variability of staining response, texture, thickness, and separation distance. The arrangement of these bands is consistent in

the individual's cells and from individual to individual in the same species. Even within separate species in one genus the interspecies banding pattern differences are usually very slight.

The organism's life history may arbitrarily begin with the first instar larvae which hatch within one to two days after the eggs are laid (15). Within a few hours after hatching the larvae begin to construct a silken case. This case has a binary purpose of protection and food trapping. The latter purpose is fulfilled after the larva has spun a food trapping net across the lumen of the case and then, by bodily undulations, causes a food bearing current to pass through the case. The larva then eats the food bearing net and proceeds to spin a replacement net.

The larvae appear in the fourth instar about 24 days after hatching, and the first adults emerge soon thereafter.

Adult females live for one and a half to two days and the males live for two or three days.

To effect fertilization, the male flies form swarms of several thousand into which the females fly and mate. The males die soon after mating has occurred. The ovipositing females are usually found on water-borne debris with their abdomens extended below the water line where oviposition occurs. An average of 804 eggs are laid per female and are embedded in long gelatinous sheaths (15,16).

The general morphology of the fourth instar larva may be described as follows:

1. Body diameter is approximately one millimeter.
2. The organism's length is approximately 15 to 18 millimeters.
3. Average dry weight of the organism is about 1.01 milligrams.
4. There are two paired salivary glands located on each side of the esophagus in the floor of the second and third somite.
5. Each salivary gland contains about 30 cells.
6. Each salivary gland contains four polytene chromosomes. The largest of these chromosomes is about 270 microns in length and about 20 microns in diameter. Two of the remaining chromosomes are somewhat shorter than the longest, while the fourth chromosome is about 70 microns in length and is terminally attached to the nucleolus.

The fourth instar larvae of Chironomus plumosus presents morphological characteristics which were considered advantageous for the purposes of this study. The polytene chromosomes of this organism offer the advantages of large size and small numbers as well as ease of handling in standard cytological methods. To summarize, the basis of selection of Chironomus plumosus may be given as follows:

1. The genus Chironomus has an extensive history as an index of stream and lake productivity, and hence is familiar to workers in this field.
2. The genus Chironomus has a world wide ecological

distribution.

3. The organism has four polytene chromosomes per nucleus, thus giving the binary advantage of few chromosomes of large size.
4. The salivary glands contain an average of 30 cells per gland which are large enough for quantitative semimicro cytological techniques.

The organisms used in this study were made available through the kindness and cooperation of Professor Troy Dorris and his staff and students of the Aquatic Biology Laboratory on the campus of the Oklahoma State University. The organisms are natural inhabitants of the fish culture tanks at the Aquatic Biology Laboratory which provided a convenient year-round source of Chironomus plumosus.

Gross Chromosome Damage Analysis

In order to determine the qualitative nature of chromosomal aberrations occurring in the experimental organisms exposed to gamma radiation, it was decided that an exploratory experiment should be carried out.

The organisms in this exploratory study were exposed to a total average dose of 18 roentgens, fractionated in doses of three roentgens per day for six days. The organisms were removed from the chamber and immediately prepared for microscopic examination at the end of the sixth day.

Since this experiment was designed to determine the qualitative nature of the gamma-ray induced aberrations, no

attempt was made to rigorously analyze the experimental results for quantitative information. A cursory quantitative examination showed an increasing trend in number of incomplete synapsis in both Chromosome II and Chromosome IV.

The types of aberrational changes induced in the chromosomes were fragments, incomplete synapsis, pycnosis, general diffuseness, and clumped sections.

The classification of chromosomal aberrations observed in the experimental organisms may be dichotomized by their generally qualitative or quantitative nature. Those of a quantitative nature may be further subdivided into three distinct types:

1. Incomplete synapsis or a "split" between the chromatid halves of a chromosome.
2. Fragments produced when a chromosome was cleaved in half somewhere along its length.
3. Clumped sections of chromosomes which appeared sometime after the organism had been removed from the irradiation chamber.

Those chromosomal aberrations which appeared to be of a qualitative nature were pycnosis and general diffuseness. A pycnotic chromosome is one which appears to have a sticky surface. This surface stickiness causes abnormal adhesion between adjacent chromosomes. The entire chromosome or only a few sections of a chromosome may be pycnotic. A general diffuse condition occurred when there is deterioration of the banded structure. Chromosomes so affected appeared to be

constructed of small oval or spherical bodies of heterochromatin dispersed in a matrix of euchromatin. This condition was usually restricted to a few major band divisions within the affected chromosome. It should be noted that any of these aberrations could simultaneously occur within the same chromosome.

Chromosomes were prepared for microscopic study by using a slightly modified version of the acetorcein technique described by Darlington and LaCour (18). The modification consisted of ignition of the ethanol vapors arising from the fixer solution during the fixing phase, in lieu of the time lapse procedure reported by Darlington and LaCour. This modified technique gave staining results as good as those observed by using the original technique and the modified procedure resulted in considerable time economy.

The Radiation Chamber

The problem of backscatter radiation is inherent in any study utilizing gamma radiation. The amount of scattering involved is a complex function of the energy of the radiation and the atomic number of the material through which the gamma radiation passes. As the energy of the gamma radiation increases from 100 Kev to 1.33 Mev the amount of scattered radiation decreases and reaches a minimum at 1.33 Mev in a low atomic number material (2,20). To minimize the scattering of gamma radiation, therefore, a low atomic number material was chosen. One quarter inch thick lucite plastic

plate, which has long been used to reduce undesirable back-scatter, was chosen as the material best suited for a radiation chamber. A sketch of the radiation chamber is shown in Fig. 1.

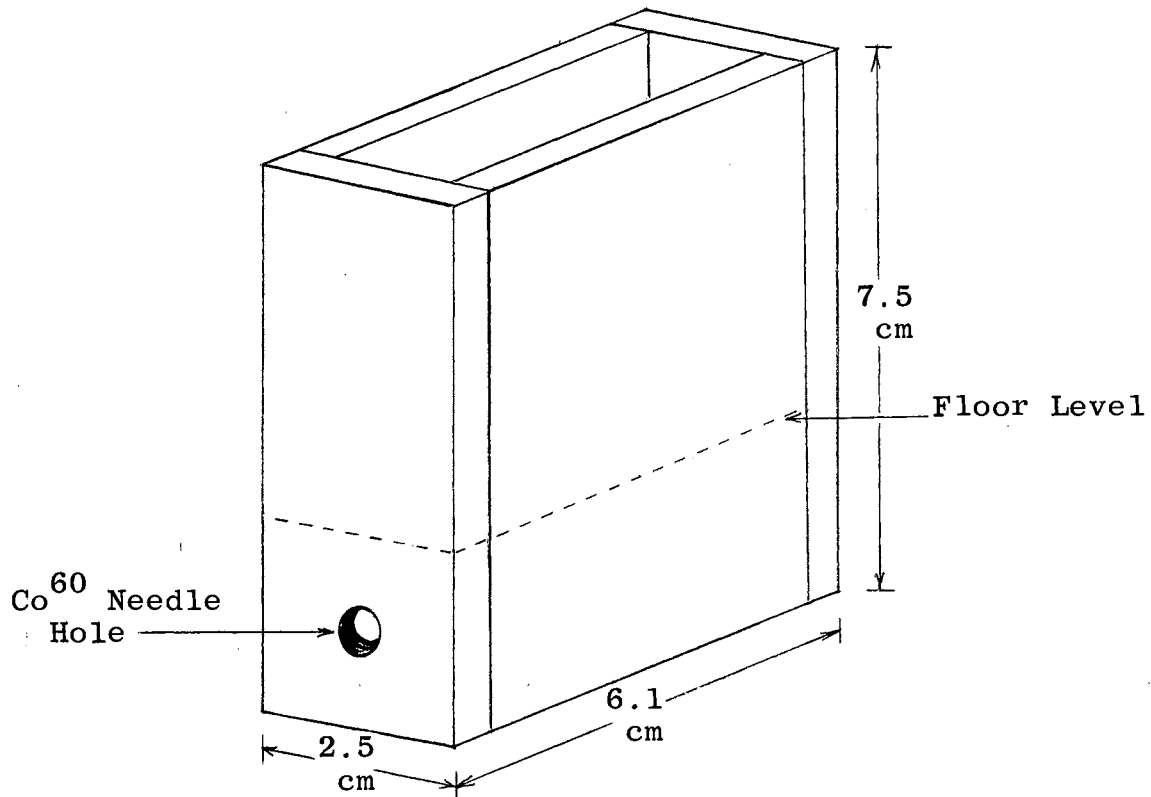


Fig. 1 Radiation Chamber

The chamber was tapped and drilled for $\frac{1}{4}$ -20 threads to match those on the Co^{60} needle. The needle was then located parallel and 1.14 cm from the central longitudinal axis of the chamber floor.

The physical dimensions of the chamber were:

1. length	6.1 cm
2. height	7.5 cm
3. inside width	1.7 cm
4. outside width	2.5 cm
5. floor thickness	0.05 mm
6. distance from the top of the chamber to the chamber floor	5.0 cm
7. distance from the chamber bottom to the chamber floor	2.5 cm

Method of Dose Delivery

The experimental organisms to be irradiated were placed in the irradiation chamber in sets of 20 larvae. Ten such sets were irradiated at ten different total exposure dose values. After irradiation was completed, ten of the most vigorous larvae were selected and processed for microscopic examination. The radiation chamber was designed to maintain the gamma flux at a constant average value of 41 roentgens per hour, thus average dose exposure was a function of time alone. The time of exposure varied from one-fourth hour to two and one-half hours, corresponding to an average dose exposure range of 10 to 102 roentgens in 10 roentgen increments. It should be emphasized that the values given above are average values based on calculated values of dose rate

at various positions on the chamber floor. In the radiation chamber floor the dose rate varied from 26.9 R/hr at the corners to 47.5 R/hr at the center. These dose rate values produce a total exposure dose range from 6.7 R to 190 R depending on time of exposure and position of the organism on the floor of the chamber. It would have been informative to have a "probability of position function" to introduce into the calculations in order to compensate for the variation of positions assumed by the organisms during irradiation, but when the purposes of this study are viewed with respect to their exploratory nature, such additional mathematical considerations seemed unnecessary.

Additional consideration might be given to the fact that the larvae habitually perform occasional vertical excursions (16,17). This would reduce the total dose absorbed by a particular larva during the irradiation time it experienced. These vertical trips were ignored during the dose calculations as their inclusion would make for prohibitively complex calculations.

The above descriptions show that there is variation of dose rate and total absorbed dose within each of the experimental studies; however, this variation was minimized by using a single radiation chamber, the same radiation source and large number of organisms. Control of the radiation dose requires exact positioning of the organism, which does not favor normal metabolic activities and serves to unduly complicate the radiation damage syndrome.

The Gamma Ray Source

An 8.54 mC Cobalt-60 needle was utilized as the gamma-ray source for irradiating the experimental animals in this study. The needle was purchased from Tracerlab, Waltham, Massachusetts. According to the manufacturer's data sheet, the source needle's strength was 13.1 mR per hour at one meter on April 18, 1963. This source strength rating corresponds to 9.7 mC of Cobalt-60, but since the source was not utilized in this study on the above date, the dose and dose rate calculations were corrected for decay according to the relationship,

$$A(t) = A_0 e^{-0.693 t/T} \quad (2-1)$$

where, $A(t)$ is the source activity after time, t , A_0 is the initial source activity and T is the half-life of the radioisotope. By setting $t = 0.9$ year and $T = 5.3$ years, and substituting these values in Equation (2-1) the source strength was found to be 8.54 mC.

Two gamma rays of 1.17 and 1.33 Mev are emitted in cascade and in equivalent amounts by Cobalt-60. This gamma ray emission is preceded by the emission of a 0.31 Mev beta particle (19). Decay to ground state by Cobalt-60 produces a stable daughter nuclide, Nickel-60. A hermetically sealed stainless steel capsule serves to contain the Cobalt-60 and also completely absorbs the beta particles such that only the gamma rays are detectable at the surface of the needle.

Gamma Ray Dosimetry

Since the gamma ray source described in the preceding section was used as a linear source rather than a point source, the calculation of dose rate and total exposed dose requires theoretical treatment of gamma ray dosimetry as well as consideration of some simplifying assumptions to make the calculations realistic.

The fundamental calculation in gamma ray dosimetry involves determination of the distribution of gamma radiation around a point source. Development of equations was made assuming that the source is surrounded by air. This assumption is valid for low energy gamma radiation at small distances from the source (20). The dose exposure around a point source follows an inverse square law, which means, in essence, that the dose exposure is attenuated exponentially as the reciprocal of the square of the distance from the source. Stated in alternate terms, the inverse square law of gamma radiation states that the dose is proportional to $1/r^2$, where r is the distance from the point source.

Gamma radiation may be characterized by its energy and abundance per disintegration of the radioisotope. For the purposes of dosimetry, these characterizing properties may be combined with the absorption coefficient for gamma rays in air. A constant disintegration rate of 3.7×10^7 disintegrations per second, will give a dose rate value at one centimeter in air from a point source which is a

characteristic constant for a given radioisotope. This dose rate constant for gamma radiation is known as the gamma ray dose rate constant, and is denoted by the upper case Greek letter Gamma, Γ (20). For a point source emitting more than one type of gamma ray, the total dose rate constant, Γ_t , may be determined by the following relationship:

$$\Gamma_t = \sum P_i \cdot \Gamma_i \cdot (E)_i \quad \text{cm}^2\text{-R/mC-hr} \quad (2-2)$$

where, P_i is the abundance, Γ_i is the dose rate constant and $(E)_i$ is the energy of the i -th gamma ray. In practice, however, since the values of Γ are subject to change as better instrumentation is developed, it is usually more satisfactory to accept the value of Γ reported by the source manufacturer. The manufacturer's value of Γ for the Cobalt-60 source utilized in this study was $13.5 \text{ cm}^2\text{-R/mC-hr}$.

The gamma radiation dose rate, Dr , at any distance r centimeters from a point source of $A(t)$ mC can be determined from the inverse square law relationship,

$$Dr(t) = \Gamma A(t)/r^2 \quad \text{R/hr} \quad (2-3a)$$

where $A(t)$ is given by Equation (2-1). Since the source strength is a function of time, Equation (2-1) is substituted for $A(t)$ in Equation (2-3a) to correct for decay. Then Equation (2-3a) becomes,

$$Dr(t) = \Gamma / r^2 A_0 e^{-0.693 t/T} \quad \text{R/hr} \quad (2-3b)$$

Since T of Cobalt-60 is 5.3 years and the irradiation times were limited to a maximum of two and one-half hours, then $T \gg t$ and Equation (2-3b) reduces to,

$$Dr = \Gamma A_0 / r^2 \quad \text{R/hr} \quad (2-3c)$$

The total dose exposure, $D(t)$, at time t , may be found by integrating over the time of exposure from $t = 0$ to $t = t$. Employing the general expression for $Dr(t)$, Equation (2-3a) then the total exposure dose is given by:

$$D(t) = \Gamma \int_0^t Dr(t) dt \quad \text{R} \quad (2-4a)$$

then,

$$D(t) = \Gamma / r^2 \int_0^t A(t) dt \quad \text{R} \quad (2-4b)$$

or

$$D(t) = \Gamma A_0 / r^2 \int_0^t e^{-0.693 t/T} dt \quad \text{R} \quad (2-4c)$$

which after integrating becomes,

$$D(t) = 1.441 A_0 T/r^2 \left(1 - e^{-0.693 t/T}\right) R \quad (2-4d)$$

For Cobalt-60, since $T \gg t$, Equation (2-4d) reduces to the simple time dependent function;

$$D(t) = \Gamma A_0 t/r^2 R \quad (2-4c)$$

A needle gamma ray source of strength A and length b may be considered as a linear array of point sources of strengths A/b (Fig. 2).

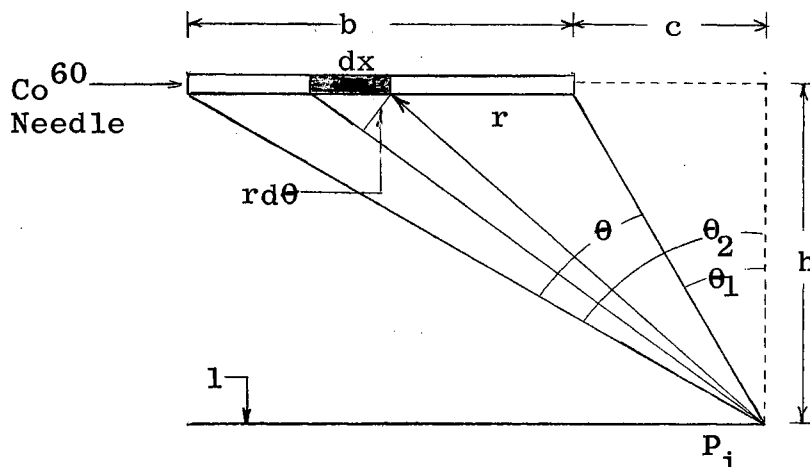


Fig. 2 Dose Rate to P_i From a Co^{60} Needle.

The gamma radiation dose rate to any point, P_i , on a line, l , parallel to the needle source may be found by integrating over the length of the needle, hence, the dose rate of P_i is

given by:

$$Dr = \int_0^a \Gamma A dx / br^2 \quad R/hr \quad (2-5a)$$

If h is the perpendicular distance from P_i to the longitudinal axis of the needle, then, $dx = rd\theta / \cos\theta$ and $r = h / \cos\theta$, and Equation (2-5a) becomes:

$$Dr = \Gamma A / bh \int_{\theta_1}^{\theta_2} d\theta \quad R/hr \quad (2-5b)$$

$$Dr = \Gamma A / bh (\theta_2 - \theta_1), \quad R/hr \quad (2-5c)$$

or

$$Dr = \Gamma A / bh\theta \quad R/hr \quad (2-5d)$$

Equation (2-5d) states that the gamma radiation dose rate at a point on a line parallel to a needle source equals the dose rate at a unit distance from a point source of strength A/b , multiplied by the angle in radians subtended by the needle, and divided by the perpendicular distance from the axis of the needle.

In theoretical treatments of gamma ray dosimetry corrections for self-absorption and source capsule absorption are usually made. In practical situations, however, one may ignore these corrections because the source strength reported by the manufacturer has already been corrected for self-absorption and capsule absorption. This procedure was

followed in this study.

Several assumptions were necessary to reduce the complexities inherent in gamma ray dosimetry. The equations defining the dose rate and total dose exposure, as well as the calculations made to determine the approximate numerical values of dose rate and dose, were based on the following assumptions:

1. The Cobalt-60 needle contains a uniform loading of A mC of Co^{60} and is b cm long.
2. The needle may be treated as n number of point sources of A/b mC each.
3. The exposure dose rate, D_r , to the i -th point, P_i , may be found by integrating over the length of the needle.
4. The equations for total exposed dose and dose rate were derived for the case of dose exposure in air. In the gamma ray energy range, $1.17 \leq E \leq 1.33$ Mev, and the distance from the source to the chamber floor range, $1.14 \leq h \leq 1.19$ cm, the dose exposure in water will not vary significantly from the dose in air.

The floor of the radiation chamber may be considered as a subset of a plane parallel to the longitudinal axis of the Cobalt-60 needle (Fig. 3).

Furthermore, the chamber floor may be thought of as a series of n lines all parallel to the longitudinal axis of the Co^{60} needle. Given the coordinates of a point, P_i , on

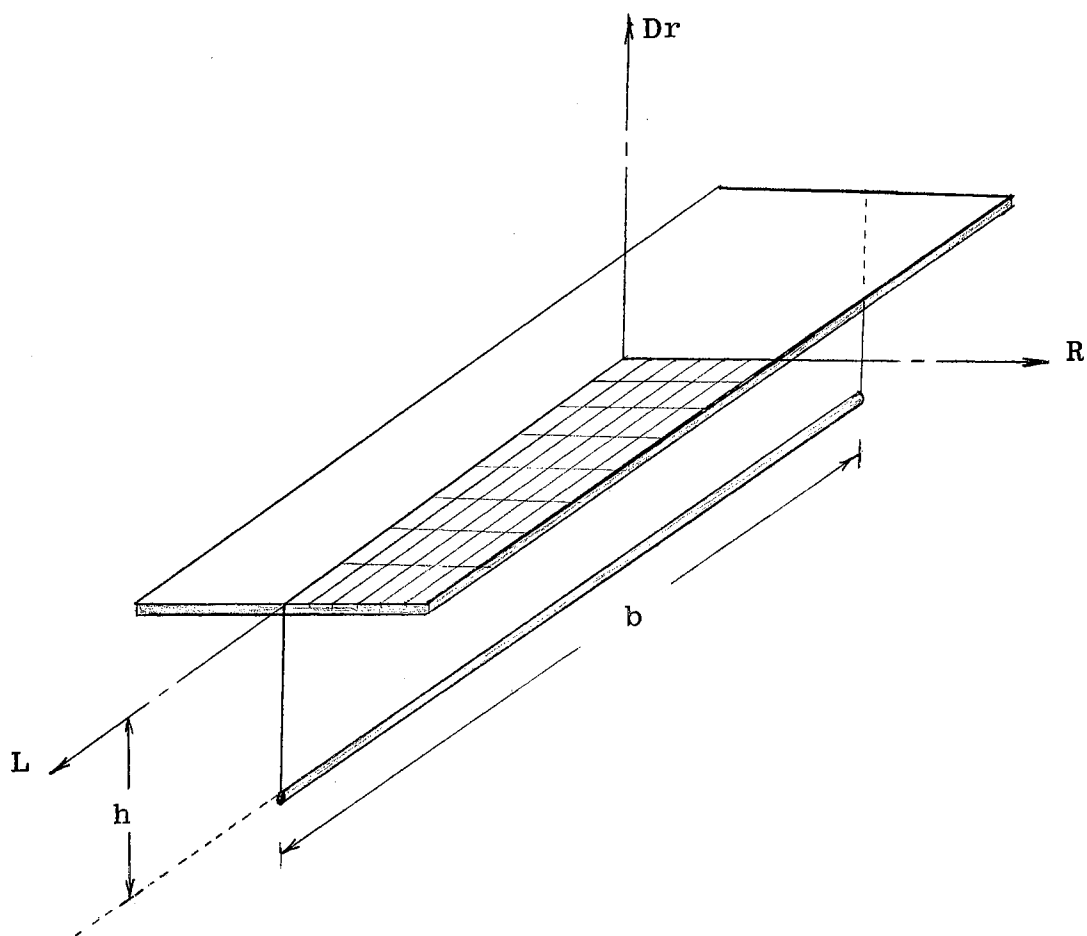


Fig. 3 Geometric Relation of Chamber Floor to the Co^{60} Needle

the chamber floor and the angle, θ , subtended by P_i and the longitudinal extremities of the needle, the dose rate to P_i may be calculated by either Equation (2-5d) or Equation (2.6). Since the chamber floor may be divided into four quadrants, symmetrical about the point (1,21), it is necessary to compute Dr for the first quadrant only. The coordinates of P_i along with the associated dose rate, Dr_1 , represents a point, B_i , given by the coordinates (L_i, R_i, Dr_i) , on a surface over the chamber floor. This surface represents the variation in Dr over the chamber floor (Fig. 4).

The dose rate calculations were made by utilizing an IBM 1620 computer and an alternate formulation of Equation (2-5d). This alternate expression is:

$$Dr = 1 A/bh \left[\arctan(b+c/h) - \arctan(c/h) \right] R/hr \quad (2-6)$$

One quadrant of the chamber floor was divided into a grid of small rectangles using 20 equal divisions of 0.12 cm each along the major axis of the floor and 10 equal divisions of 0.03225 cm each along the minor axis of the floor (Fig. 3). The dose rate was computed for each of the 231 intersection points of the grid. The average dose rate was computed using the trapezoidal rule; that is, the values of the rates were assigned relative values of one at each of the four corners, two at each of the other 56 perimeter points, and four at each of the 171 interior points. Each computed dose rate and the average dose rate was determined to a precision of three significant figures, this being the precision of

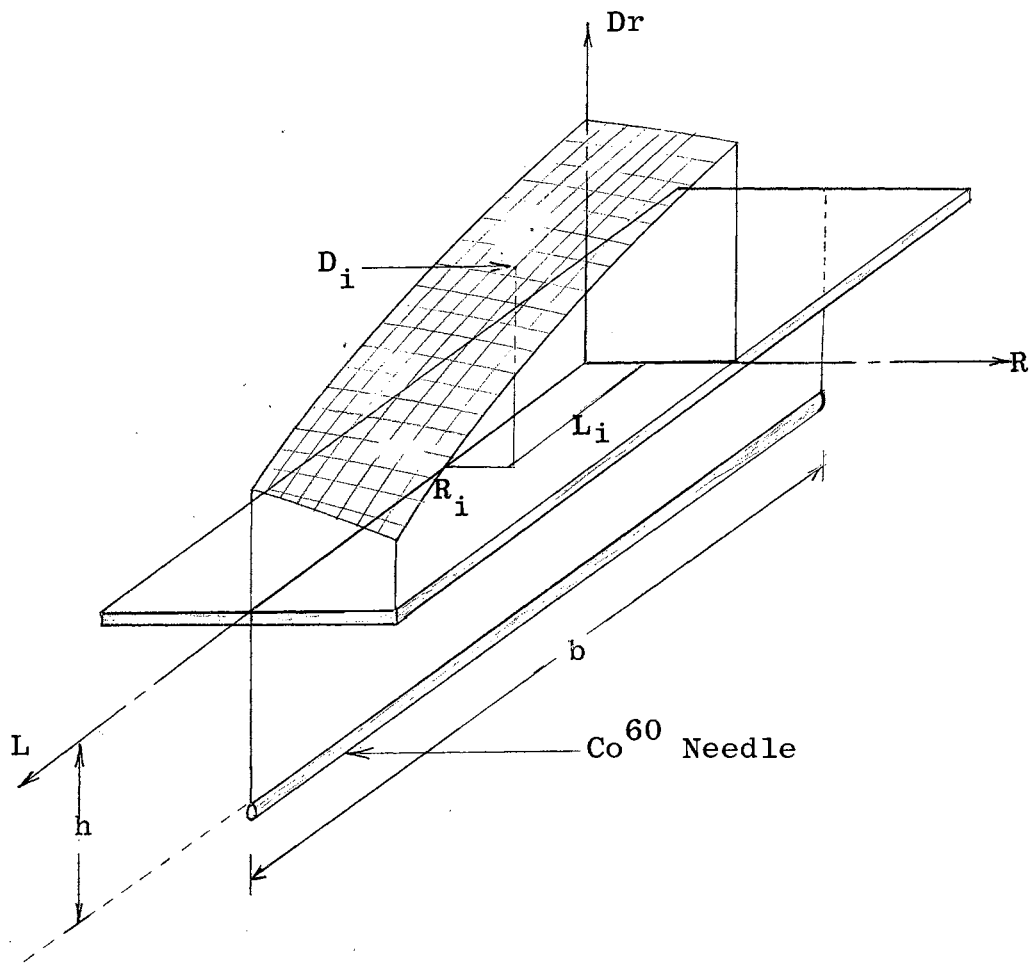


Fig. 4 Surface Representation of Dr Variation Over Quadrant I of the Chamber Floor

the dimensions and radiation constants. Isodose lines for Quadrant I of the radiation chamber floor are shown in Fig. 5. Both the chamber floor and associated isodose lines are symmetrical with respect to the geometric center of the floor, hence only the first quadrant values have been shown. The center of the floor is represented by the upper left hand corner of Fig. 3 and the coordinates $L = 1$ and $R = 21$ specify this position on the floor. The coordinates $L = 11$ and $R = 1$ represent the corner of the floor diagonally opposed to the center. The dose rate had a maximum value of 47.5 R/hr at the center and a minimum value of 26.9 R/hr at the above mentioned corner of the chamber floor.

The computed values of Dr over Quadrant I of the chamber floor are listed in Table I. Values are listed according to corresponding line and row coordinates. For example, the center of the floor is represented by the point intersected by $L = 1$ and $R = 21$, and Dr has a value of 47.5 R/hr at the center. At the point represented by $L = 4$ and $R = 16$, Dr has the value 46.4 R/hr. A total of 231 dose rate values are listed in Table I, and since the four quadrants of the chamber are symmetrical about the center, isodose lines may be developed for a total of 924 points on the chamber floor.

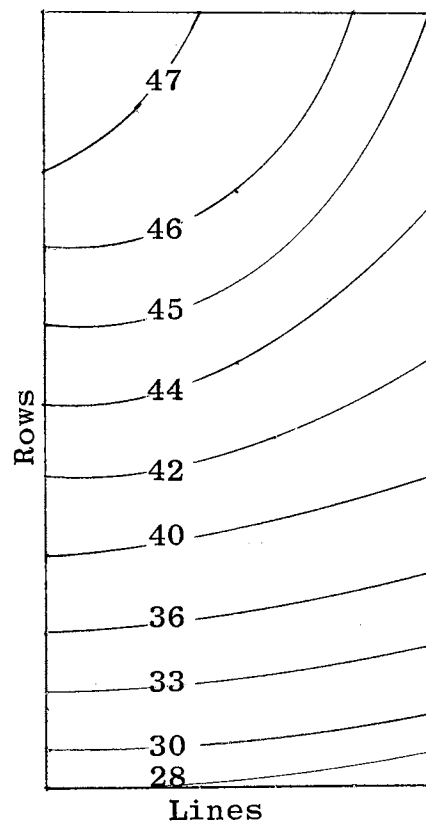


Fig. 5 Isodose
Lines for Quadrant
I of the Chamber
Floor

TABLE I

VALUES OF DOSE RATE AT 231 POINTS ON QUADRANT I OF THE RADIATION CHAMBER FLOOR

R \ L	1	2	3	4	5	6	7	8	9	10	11
1	28.1	28.1	28.1	28.0	27.9	27.8	27.7	27.5	27.3	27.1	26.9
2	30.2	30.2	30.2	30.1	30.0	29.9	29.7	29.7	29.3	29.1	28.8
3	32.3	32.2	32.2	32.1	32.0	31.8	31.7	31.5	31.2	31.0	30.7
4	34.2	34.2	34.1	34.0	33.9	33.7	33.6	33.3	33.1	32.8	32.5
5	36.0	36.0	35.9	35.8	35.7	35.5	35.3	35.1	34.8	34.5	34.2
6	37.7	37.6	37.6	37.5	37.3	37.2	36.9	36.7	36.4	36.1	35.7
7	39.2	39.2	39.1	39.0	38.8	38.6	38.4	38.1	37.8	37.5	37.1
8	40.5	40.5	40.4	40.3	40.2	40.0	39.7	39.5	39.1	38.8	38.4
9	41.7	41.7	41.6	41.5	41.3	41.1	40.9	40.6	40.3	39.9	39.5
10	42.8	42.7	42.7	42.5	42.4	42.2	41.9	41.6	41.3	40.9	40.5
11	43.7	43.6	43.6	43.5	43.3	43.1	42.8	42.5	42.2	41.8	41.4
12	44.5	44.4	44.4	44.2	41.1	43.9	43.6	43.3	43.0	42.6	42.2
13	45.1	45.1	45.0	44.9	44.7	44.5	44.3	44.0	43.6	43.2	42.8
14	45.7	45.7	45.6	45.5	45.3	45.1	44.8	44.5	44.2	43.8	43.4
15	46.2	46.2	46.1	46.0	45.8	45.6	45.3	45.0	44.7	44.3	43.8
16	46.6	46.6	46.5	46.4	46.2	46.0	45.7	45.4	45.0	44.6	44.2
17	46.9	46.9	46.8	46.7	46.5	46.3	46.0	45.7	45.3	45.0	44.5
18	47.2	47.1	47.0	46.9	46.7	46.5	46.3	45.9	45.6	45.2	44.8
19	47.3	47.3	47.2	47.1	46.9	46.7	46.4	46.1	45.8	45.4	44.9
20	47.4	47.4	47.3	47.2	47.0	46.8	46.5	46.2	45.8	45.5	45.0
21	47.5	47.4	47.4	47.2	47.0	46.8	46.6	46.2	45.9	45.5	45.0

* L = line; R = row; Total number of points represented = 231

CHAPTER III

EXPERIMENTAL RESULTS

The Control Population Survey

A survey of the chromosome arrangement and appearance in the natural population of Chironomus plumosus was made in order to determine the types and frequencies of naturally occurring chromosomal aberrations. This control survey was conducted over a period of four months and included members from three subsequently occurring generations in order to determine if this species of Chironomus exhibited variability of chromosome structure from generation to generation. Variability of chromosome structure of this nature, as well as variability between members of the same generation of organisms, has been reported by Action (21-23) from the results of observations made on populations of Chironomus tetans and Chironomus dorsalis inhabiting ponds in the United Kingdom and Canada.

Results of this survey showed that this particular population of Chironomus plumosus displayed very consistent chromosome structure. A total of 2,048 chromosomes representing 512 full nuclear complements of four chromosomes per nucleus were observed during the control survey. Since this

sample displayed a high degree of consistency, it was decided that this was an adequate sample size for the purposes of this study.

The results of the control population are listed in Table II. Chromosome I and Chromosome III did not display a single aberration in those examined from blood worms of the control populations. Chromosome III did tend to form Balbiani rings, however, according to Beermann (12), Balbiani rings are not to be considered aberrational in nature. Within the 512 Chromosome III's observed, 180 showed incomplete synapsis or 35.2 per cent of these chromosomes displayed the type of aberration known as incomplete synapsis. These 180 splits account for 8.78 per cent of the total percentage of aberrations observed in the natural population. Similarly, 23 of the 512 examined Chromosome II's displayed incomplete synapsis, or 4.49 per cent. These 23 aberrations account for 1.12 per cent of the total 9.91 per cent chromosomes exhibiting incomplete synapsis.

No fragmented chromosomes were observed within the control population.

Results of Varying Total Dose

These experiments were designed to determine if the chromosomes of the experimental organism exhibited a degree of consistent response to radiation which would allow them to be used as a biological radiation dosimeter.

The organisms were subjected to radiation doses ranging

TABLE II
 CHROMOSOMAL ABERRATIONS OBSERVED IN CONTROL POPULATION*
 CHIRONOMUS PLUMOSUS

Organism Number	Number of Chromosomes Observed	Number of Incomplete Synapsis	% Incomplete Synapsis
1	64	6	9.4
2	64	7	10.9
3	68	5	7.4
4	128	15	11.7
5	144	14	8.3
6	76	5	6.6
7	80	13	16.2
8	112	12	10.7
9	108	14	13.0
10	124	22	17.7
11	100	9	9.0
12	128	11	8.6
13	120	8	6.7
14	80	6	7.5
15	124	12	9.7
16	100	8	8.0
17	92	10	10.9
18	152	12	7.9
19	80	8	10.0
20	104	6	5.8
Total	*2,048	203	9.91

from 10 - 100 R in increments of 10 R. The dose rate was held at a constant maximum value of 41 R/hr.

It was necessary, because of time restrictions, to use two generations of experimental organisms in this experiment. The first experimental population covers the range of total dose from 10 - 51 R and the second experimental population covers the range of total dose exposure from 61 - 102 R. In view of the possibility of variation of response to gamma radiation between generations, it was assumed that some error may have been introduced by the use of two experimental populations.

The predominantly observed aberrations in the range from 10 - 72 R were fragments and incomplete synapsis. Aberrations such as pycnosis and diffuseness were relatively sparse and completely disintegrated and clumped nuclei did not appear until the dose levels were increased to 82 R.

In the range from 0 - 41 R total dose, per cent fragments showed a trend toward a linear response and this linear trend broke down after 41 R.

In the range from 82 - 102 R, the appearance of many cases of completely shattered and clumped nuclei made interpretation of results difficult. For example, out of 640 chromosomes observed in the 102 R dose level, 56 chromosomes from 14 nuclei showed such a degree of destruction as to make clear observation impossible.

The first set of ten experimental organisms were exposed to a total dose of 10 R. The organisms were immediately

sacrificed and the salivary gland cells of the organisms were processed for examination as described in the chapter on experimental methods. Salivary glands from two organisms were placed on each of five slides, and a total of 628 chromosomes were observed. Of these 628 chromosomes, an average of 12.4 per cent showed some degree of incomplete synapsis or "splits". The range of per cent incomplete synapsis was between 12 and 13.6 per cent. An average of 2.03 per cent of the observed chromosomes showed some degree of fragmentation and it is to be recalled that the control population did not exhibit this type of aberrations. The range of fragments from the five slides ranged between 0.93 and 2.35 per cent.

The second set of organisms was exposed to a total of 20 R. An average of 14.1 per cent of the observed chromosomes exhibited incomplete synapsis and the range of "splits" lay between 8.6 and 17.4 per cent. The average observed fragments was 3.74 per cent with a range of 2.34 - 5.3 per cent. A total of 676 chromosomes was observed from this group of organisms.

The third group of organisms was exposed to a total of 31 R and 620 chromosomes were observed. In this population, 16.5 per cent of the chromosomes showed splits with a range between slides of 16 and 17.9 per cent. An average of 6 per cent of the chromosomes was fragmented and the range between slides was 4.45 and 7.2 per cent. To this point, 31 R total dose, the per cent "split" chromosomes tended to show a

linear response with increase in total dose, an increase of approximately 2 per cent for each 10 R incremental increase in total dose. Chromosome damage in the 41 R group of organisms do not show an obvious patterned response to increased dose. This is particularly obvious with regard to the induction of incomplete synapsis.

A total of 624 chromosomes was observed in the fourth set of experimental organisms. These organisms were exposed to a total dose of 41 R. An average of 12 per cent of the chromosomes observed in this experiment exhibited incomplete synapsis. Variation in the incomplete synapsis values ranged from 10.2 to 13.3 per cent. Fragments appeared in about 8.05 per cent of these chromosomes and ranged from 7.01 per cent to 13.2 per cent. The relationship between fragments produced and radiation absorbed is approximately linear.

The fifth set of organisms was exposed to 51 R and 600 chromosomes were recovered for examination. An average of 15.1 per cent of these chromosomes exhibited incomplete synapsis with a range of 13.7 to 16.7 per cent between the members of the set. An average of 7.34 per cent of the observed chromosomes were fragmented and the values ranged between 5.3 per cent and 13.2 per cent, a difference of 7.9 per cent units between the extremes of the range of values.

Experiments involving sets one through five of the experimental organisms were conducted over a period of about three weeks. By the end of the third week the condition of

the population, from which the experimental organisms were secured, had degenerated to such a degree that there were no remaining fourth instar larvae suitable for use as experimental organisms. A new population of organisms was secured and sets six through ten were members of this second population of Chironomus. As previously mentioned, comparisons between the responses of various sets of organisms to radiation should be made with the realization that there may be variation in radiosensitivity between populations of the same organism.

The sixth set of experimental organisms was exposed to 61 R and 612 chromosomes were recovered for examination. An average of 8.15 per cent of these chromosomes displayed incomplete synapsis, and the values between pairs of individuals or organisms within the set ranged from 6.24 to 9.84 per cent. An average of 3.24 per cent of these chromosomes displayed fragments with a range of 2.27 to 4.7 per cent within the set. The data from this experiment fell below the expected values when comparison is made with data obtained from prior experiments in this study. These results are to be considered questionable and are included here only to illustrate the variation in results obtained using the visual experimental method on which this study was based.

An average of 17.9 per cent of the 652 chromosomes examined in the seventh group displayed incomplete synapsis. The values, within the set, between individual pairs of

organisms ranged from 14.6 to 21.6 per cent. The organisms were exposed to 72 R prior to examination. The chromosome damage averaged 16.9 per cent fragments and these values ranged from 14.1 per cent to 20.5 per cent.

Beginning with the eighth set of organisms, which had been exposed to 81.8 R and including the remaining two sets of organisms, it became increasingly more difficult to determine the nature and extent of the damage to the chromosomes induced by the radiation exposure. This difficulty was experienced because the number of nuclei, and included chromosomes, was damaged beyond recognition.

The eighth group of experimental organisms was exposed to 81.6 R and 592 chromosomes were recovered for observation and analysis. An average of 15.5 per cent of these chromosomes exhibited a degree of induced incomplete synapsis with a range of 14.9 to 16.7 per cent between group pairs of organisms. Fragmented chromosomes ranged from 11.3 to 22.0 per cent between group pair members, with the average being 14.1 per cent.

The ninth group of organisms was exposed to 90 R and 584 chromosomes were recovered for observation. An average of 22.2 per cent of these chromosomes was split and the values between pairs ranged from 21.5 to 24.2 per cent. The induced fragments averaged 20.6 per cent and ranged from 10.4 to 25 per cent.

The tenth group of experimental organisms was composed of only eight members due to the fact that only eight

appeared to be in the same vigorous state as those organisms in the other nine groups. These organisms were exposed to 102 R and 640 chromosomes were recovered for observation. As previously mentioned, a total of 56 chromosomes was destroyed to the degree that it was impossible to classify the damage they exhibited. Of the remaining chromosomes, an average of 17.8 per cent displayed incomplete synapsis with values ranging from 14.7 to 21.2 per cent among the group members. Fragments averaged 17.45 per cent and ranged between 14.3 per cent and 22.1 per cent. These values of induced aberrations would possibly have been significantly higher if it had been possible to analyze the 56 completely destroyed chromosomes.

The results of this study indicate that the use of Chironomus as a biological radiation should be based on the induction of chromosomal fragments if a visual method of analysis is deemed desirable. The results presented here indicate that incomplete synapsis exhibit too large a degree of variability to make extrapolation from biological effect to absorbed radiation dose quantitatively possible.

The visual method of analysis presents several serious considerations, including the amount of time required to prepare organisms for examination, and the length of time necessary for examination. These considerations limit the sample size which can be handled by a single operator. A measure of subjectivity is also inherent in the visual analysis, particularly as to what constitutes a damaged

chromosome.

Direct comparison of the results of this experimental study are provided in graphic form in Fig. 6 and are listed in Table III. These results show that there is a general increase in chromosome damage with increasing doses of gamma radiation. In general, the points beyond the 41 R dose show significant variation. In the range from background to 41 R each of the three curves, fragments, incomplete synapsis and total aberrations show a well defined linear trend upward without excessive variation. Past the 41 R point, there is extreme variation in all points; however, the variation is greatest in the fragment curve. The large variation in chromosome damage exhibited by the large radiation dose was predicted above. The condition of the chromosomes following radiation doses greater than about 40 R destroys the integrity of the structure and alters the staining characteristics of the structures. In chromosomes receiving less than 40 R radiation dose there is small loss of chromatin material and the response to stain is not changed from the normal. This finding shows that the aberrations produced by large doses of radiation, greater than 40 R, are not linear and are difficult to place in quantitative order. The change in response of the system at the 40 R point suggests application of this system as a threshold gamma radiation dosimeter.

The consistent and smooth linear relationship exhibited in the lower dose range, background to 41 R, shows that it might be possible to extend the range of the damage system

TABLE III

CHROMOSOMAL ABERRATION OBSERVED IN THE IRRADIATED
POPULATIONS OF CHIRONOMUS PLUMOSUS

Organism Set Number	Number of Chromosomes Observed	Number of Fragments	Number of Splits	Total Aberrations
1	132	3	18	21
2	128	2	16	18
3	132	4	16	20
4	108	1	13	14
5	128	3	15	18
<u>Totals</u>	<u>628</u>	<u>13</u>	<u>78</u>	<u>91</u>
Total Dose=10.2 R; % Frag=2.07; % Splits=12.4; % Aber=14.5				
1	132	7	23	30
2	120	5	18	23
3	132	4	20	24
4	128	3	11	14
5	156	6	16	22
<u>Totals</u>	<u>668</u>	<u>25</u>	<u>88</u>	<u>113</u>
Total Dose=20.5 R; % Frag=3.74; % Splits=13.2; % Aber=16.9				
1	128	7	20	27
2	128	6	20	26
3	128	8	23	31
4	120	8	19	27
5	116	7	22	29
<u>Totals</u>	<u>620</u>	<u>36</u>	<u>104</u>	<u>140</u>
Total Dose=30.7 R; % Frag=5.8; % Splits=16.8; % Aber=22.6				
1	132	10	16	26
2	120	8	10	18
3	128	9	13	22
4	128	8	17	25
5	128	9	16	25
<u>Totals</u>	<u>636</u>	<u>44</u>	<u>72</u>	<u>116</u>
Total Dose=40.9 R; % Frag=6.9; % Splits=11.3; % Aber=18.2				
1	132	9	19	28
2	124	10	20	30
3	128	6	22	28
4	128	5	20	25
5	132	9	18	27
<u>Totals</u>	<u>644</u>	<u>39</u>	<u>99</u>	<u>138</u>
Total Dose=51.1 R; % Frag=6.05; % Splits=15.4; % Aber=21.4				

TABLE III Continued

Organism Set Number	Number of Chromosomes Observed	Number of Fragments	Number of Splits	Total Aberrations
1	128	6	12	18
2	132	3	13	16
3	144	7	5	17
4	128	3	3	11
5	80	3	66	9
<u>Totals</u>	<u>612</u>	<u>22</u>	<u>44</u>	<u>66</u>
Total Dose=61.4 R; % Frag=3.59; % Splits=7.18; % Aber=10.8				
1	128	18	22	40
2	108	17	16	33
3	164	28	24	52
4	116	24	25	49
5	136	23	29	52
<u>Totals</u>	<u>652</u>	<u>110</u>	<u>116</u>	<u>226</u>
Total Dose=71.6 R; % Frag=16.9; % Splits=17.8; % Aber=34.7				
1	128	17	19	36
2	116	18	18	36
3	160	18	24	42
4	68	15	11	26
5	120	15	20	35
<u>Totals</u>	<u>592</u>	<u>83</u>	<u>92</u>	<u>175</u>
Total Dose=81.6 R; % Frag=14.0; R Split=15.5; % Aber=29.6				
1	124	25	27	52
2	124	30	28	58
3	96	24	23	47
4	124	29	30	59
5	116	12	25	37
<u>Totals</u>	<u>584</u>	<u>120</u>	<u>133</u>	<u>253</u>
Total Dose=92 R; % Frag=20.5; % Splits=22.8; % Aber=43.3				
1	256	46	44	90
2	252	36	37	73
3	132	21	23	44
<u>Totals</u>	<u>640</u>	<u>103</u>	<u>104</u>	<u>207</u>
Total Dose=102.3 R; % Frag=16.1; % Splits=16.3; % Aber=32.3				

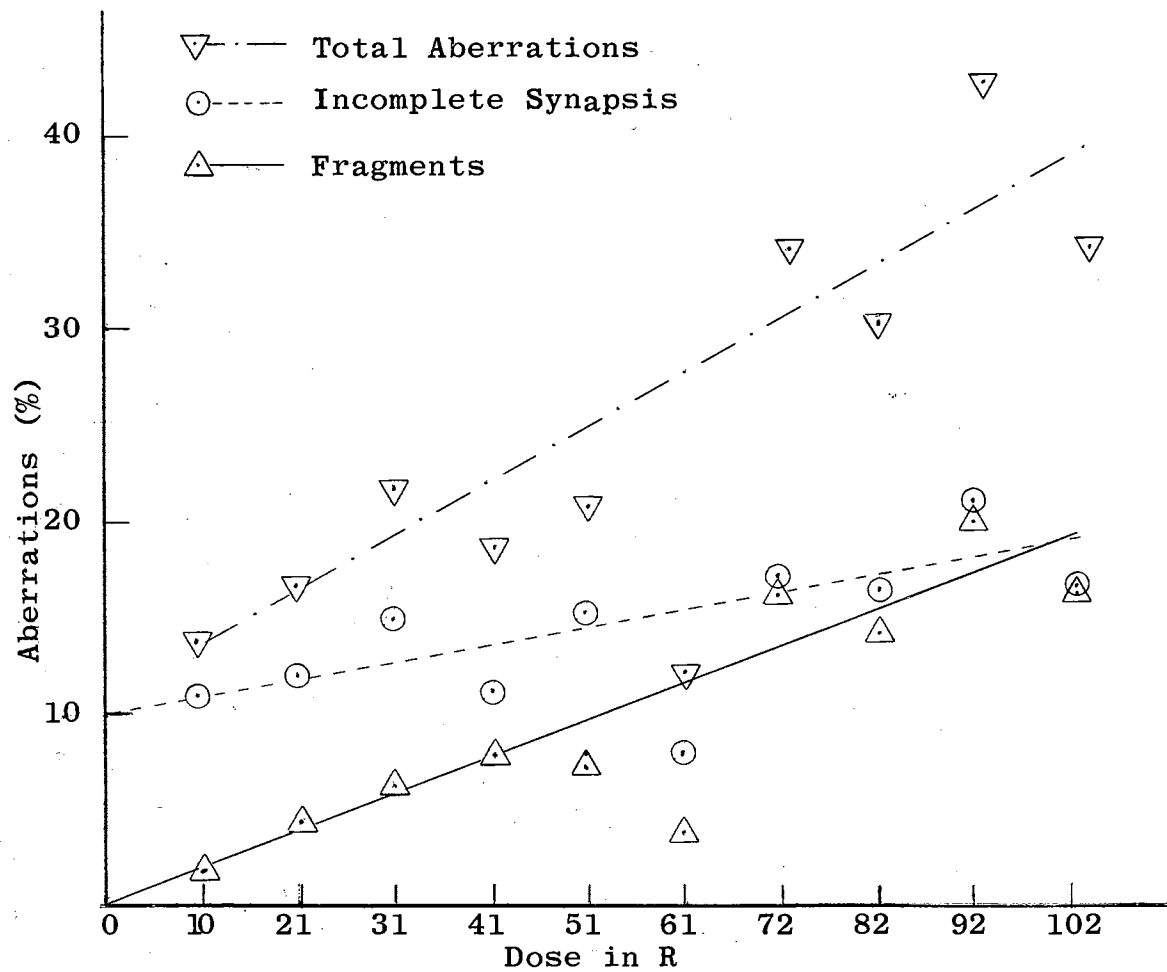


Fig. 6 Aberrations Observed in the Irradiated Population of Chironomus

to low radiation levels. The linear response of the low dose samples is in agreement with results of other studies. In addition, these results indicate that variations in other chromosome-damage-by-gamma-radiation studies may not be related to chromosome restitution.

Time Delay Study

In the preliminary radiation experiment several instances of clumped or intertwined chromosomal segments were observed. Moreover, the percentage of incomplete synapsis and fragments was greater than expected for a total dose of 18 R. It was therefore decided to determine if there was a delayed response effect. The results of this study are listed in Table IV.

TABLE IV
INCREASE IN CHROMOSOMAL ABERRATIONS AS A
FUNCTION OF POST-IRRADIATION TIME

Post Irrad. Time (days)	Fragmented Chromosomes %	Incomplete Synapsis %	Clumped Sections %	Number of Chromosomes Observed
0	15.2	16.9	0	616
1	17.2	20.2	5.7	608
2	20.3	20.7	10.4	636
3	18.0	22.2	15.4	644
4	19.0	22.3	15.6	620

Total Number Chromosomes Observed = 3,124

Experimental procedure for this study was designed such that the experimental organisms were exposed to a total average dose of 82 R. Ten of the organisms were immediately sacrificed and examined for aberrations in order to establish a reference line. On each of the next four successive days ten organisms were examined for any increases in aberrations.

The results of this study show that there is a definite trend for fragments and incomplete synapsis to increase with time after irradiation of the organisms. In addition, these results show that a discrete period of time must lapse before the clumped section aberrations appear.

Comparison of the chromosome damage-time delay values, Fig. 7, shows that fragments tend to increase for two days after irradiation and then reach a maximum and remain constant thereafter. It should be remembered that although the data show a slight variation from the third day to the fourth and fifth days, polytene chromosomes do not exhibit restitution of aberrations. This means that, once a break is induced, it is a permanent event. It is, therefore, obvious that fragments tend to become constant after sufficient time. Similar results were observed with incomplete synapsis. These aberrations increased until the fourth day and then tended to remain at the same level.

Clumped chromosomal sections appeared on the first day after irradiation and increased in a linear fashion until the fourth day. The number of clumped sections observed on

the fifth day were only slightly greater than the number observed on the fourth day. The experiment was terminated after the fifth day because the organisms had advanced into the pupal stage and there were insufficient number of fourth instar larvae remaining.

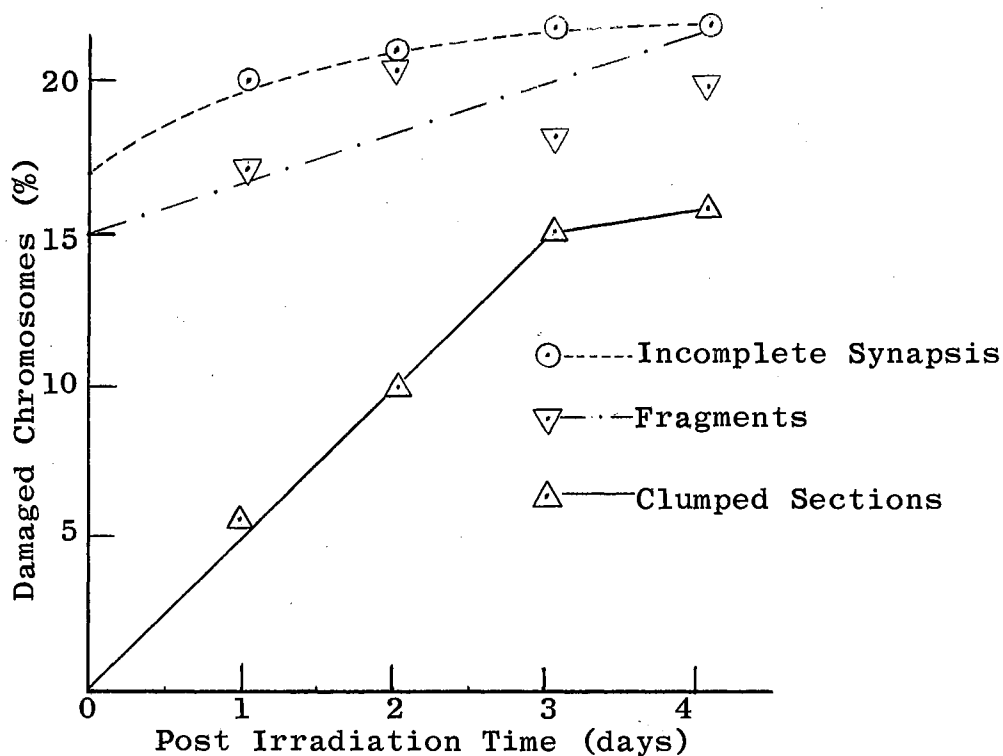


Fig. Increase in Chromosomal Aberrations

The results of the time delay study indicate that the target theory of biological response to radiation is not sufficient to explain these responses. Rather, it would

seem that there exists at least a second mechanism of radiation induced damage which requires the passage of an undetermined amount of time in order to express itself as a biological consequence to radiation exposure. The "diffuse theory", a rather ill defined and vague theory at best, holds that a direct "hit" or energy transfer to the target is not necessary in order to produce a particular biological response to radiation. This theory does not offer any particular mechanism of action; however, on the basis of chemical responses to radiation, one might suppose the mechanism would lie within the realm of the production of free radicals by ionizing radiation within the biological system, and the subsequent interaction of the free radical with the chemical systems within the living cell. Any treatment of these interactions would require the understanding of the biochemical processes within the cell and, at this time, the biochemistry of even a small subsystem of the simplest cell is only vaguely understood.

As a result of this experiment dealing with the delayed expression of induced chromosomal damage, any future application of such a system as a detector of radiation damage, should include correction for possible delayed effects. This correction would presume a calibration of the chromosomal system for increase in damage with respect to time. The results of this experiment indicate that the expression of delayed effects reached a maximum on the third day after irradiation. The organisms were, by this time, entering the pupal stage and

thus probably experiencing a radical change in cellular chemistry. This normal chemical metamorphosis would have some effect on the production of aberrations.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Summary

This study was designed to determine if the chromosomes of Chironomus plumosus displayed a quantitative response to radiation damage which would allow their use as a biological detector of radiation absorbed dose.

The results of this study are considered inconclusive over the range of total dose from 51 to 102 R. The results of dose to chromosomal response display too great a variation to determine if a visual method of analysis could be utilized at these dose levels. Similar comments can be directed toward the results obtained over the range from 10.2 to 40.1 R in the case of incomplete synapsis. This chromosomal aberration showed a generally increasing trend, but exhibited a high degree of variation with regard to per cent response to incremental increases in total absorbed doses.

In the range from 10.2 to 40.9 R, fragmented chromosomes displayed a near linear response. A incremental increase of 10.2 R in total dose exposure resulted in a corresponding increase of approximately 2 per cent of chromosomal fragmentation.

Conclusions

One obvious conclusion resulting from this study is that in order to develop an adequately quantitative biological dosimeter using a chromosomal system as a detector, a more sensitive method for damage analysis must be developed. It may be impossible to put a visual method, such as used in this study, on a sufficiently quantitative basis to meet the demands of an accurate biological dosimeter. Perhaps a better approach to this problem would develop from the analysis of the damage response of the bases on a chromosomal DNA molecule. Another possibility would involve the changes in enzyme activity with increases in radiation absorbed dose. At least one pair of workers, D. A. Rappoport and R. R. Fritz (24), studying the response of whole body radiation on the inactivation of nucleoside phosphorylase in rat erythrocytes, report that the dose-response curves obtained are suitable for predicting doses from unknown situations. Furthermore, these investigators show that the relationship between gamma radiation dose and per cent inactivation of the enzyme is linear.

Another criticism which can be made of the visual technique is that it completely ignores the possibility of latent or delayed effects. This might be overcome by the use of biochemical methods in which the chemical cause of latent effect could be determined at the time of analysis. Hadorn (25-27) reports that in mutants of Drosophila

melanogaster the quantitative and qualitative spectrum of a group of pigments, pterins, is significantly different in the mutants when compared to a normal fly's pterin spectrum. It might be well to study the change in the pigment spectrum of irradiated versus normal Chironomus larvae, in order to determine the feasibility of their application as a radiation absorbed dose detecting system.

In spite of the limitations inherent in this study, the experimental results show a definite overall trend toward linearity of response to variation in total radiation exposure dose. This would indicate that this organism would serve well in a qualitative dosimetric role in contaminated environmental situations. With refinements in analytic techniques, the organism may serve in a quantitative or semi-quantitative capacity as well.

Extensions

Assuming major revisions in techniques, an obvious extension of this study would be the comparison of internally injected gamma radiation induced damage with the results obtained in this or similar study. Moreover, since radiation effects at a given total dose vary with the energy of the gamma or x-rays, another extension of this study might include the consideration of variation in damage by varying the energy range of the photons while holding dose rate and total dose constant. This variation in photon energies would include both internally and externally delivered doses.

With regard to alpha radiation, one would be forced to consider only internally injected monoenergetic alphas, at variable total dose, and variable energetic alphas injected internally, at constant dose, because alpha radiation is quite limited in its ability to penetrate even thin layers of most materials.

The consideration of neutron induced damage should include high energy as well as thermal neutrons. Neutron doses would, in all probability, have to be limited to external dose only, because water soluble neutron emitters are not available. The neutron source should be free from gamma contamination and this would limit the choice of neutron source to either reactors or a stationary Pu^{239} - Be neutron howitzer. The latter source will give a flux of about 1.4×10^6 neutrons per cm^2 per second per curie, with neutron energies ranging from thermal energy to 10.6 Mev (2). For a greater variety of both energies and flux, one would have to utilize a nuclear reactor.

To study the damage induced in chromosomes by high energy particles, one must consider the utilization of high energy particle accelerators. The type of accelerator used would depend on the type of particle desired.

As a culmination of the above suggestions, consideration of mixed radiations and their effects on chromosomes might be considered as the ultimate goal. The Health Physicist would welcome a direct method of determining the biological effects of mixed radiations based on the types and per cent

abundance of the various components of a mixture of radiations, because the quantitative prediction of the ultimate effect of mixed radiation exposure to living systems would become possible.

With the advent of the space age, it became immediately obvious that the effect of extraterrestrial radiation sources, such as the Van Allen belt, would be added to the genetic mutation pool of mankind. Manned space vehicles would be subjected to types and intensities of radiation heretofore unexperienced by man. It would be of significant future, as well as present, interest to determine the nature and extent of chromosomal damage induced by space radiations. Since Chironomus has previously been nominated as an ideal terrestrial environmental biological radiation indicator, it may be wise to consider the use of this organism as an analogous indicator in the case of extraterrestrial radiation as well. Using this organism in all feasible cases would offer consistency in extrapolation between the effects of radiation from a variety of sources.

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