GENE NUMBER, KIND, AND SIZE IN DROSOPHILA

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The literature on X-ray effects contains two fundamental ideas which, if combined, should offer a better understanding of the physical properties of the gene on the one hand, and of the mechanism by which X-rays affect living tissue on the other. The first of these ideas is due to CROWTHER. He interpreted the rates at which living cells are killed by X-rays as due to the destruction of a vital center within each of these cells. The second idea comes from MULLER'S demonstration that X-rays impinging on chromatin will produce new gene mutations which, in turn, lead to new heritable characters. In order that the problem may be sharply defined, we shall confine the discussion to data bearing upon the kind and size of the genes present in an animal organism. The approach lies in a knowledge of the physical characteristics of X-rays in relation to their biological effects and to the rates at which these alterations are produced. This paper presents an analysis of this problem on data obtained from the treatment of Drosophila sperm with long wave-length X-rays of known energy. Sperm of the wild-type were chosen since they represent concentrated chromatin whose full effects under normal conditions are better understood than those of any other material.

MATERIALS AND METHODS

The experiment utilizing the copper X-ray was performed first. Wild-type males and females taken from a stock which had been kept and studied in the laboratory for many years were mated. For the first cultures, 4 or 5 pairs were used to a bottle. This practice proved to be undesirable as the resulting progeny were crowded and somewhat reduced in size. In later work only pair mating was practiced. The male progeny, about 30 hours old, were placed in Petri dishes 1 inch in diameter, about $\frac{1}{8}$ of an inch deep, and covered with silk bolting cloth. These dishes and their contained males were placed in the X-ray beam from 30 to 240 seconds to give the requisite X-ray dosage. (The X-ray exposures in these experiments were made in the laboratory of DR. RALPH W. G. WYCKOFF. The writers are indebted to him for the use of his apparatus, for calculation of the X-ray ionization values, and for his advice and counsel.)

The previously described gas tube (WYCKOFF and LAGSDIN 1930), equipped with targets of different metals, has been used to obtain the intense beams of X-rays needed for these experiments. These beams con-

sisted mainly of the K lines of copper and chromium, depending on the metal which was used as the target. Because of the loss of intensity consequent upon filtration and the desirability of having the data with the different radiations as comparable as possible, no attempt has been made to render the beams in any of these experiments more nearly monochromatic. Other data on filtered and unfiltered copper rays indicate, however, that only a moderate error is introduced by this slight heterogeneity.

The current through the X-ray tube during the irradiations was held constant at 4 milliamperes. The peak voltage was 34 K.V. as measured with a 12.5 cm sphere gap.

With the soft rays from copper and chromium it is necessary to make a correction for the amount absorbed in the layer of air between the surface of introduction and the volume giving the ionization current. The length of this air column was 3.2 cm. Taking the absorption coefficient (KAYE 1923) of Cu K radiation as $\mu/P = 8.43$ and of Cr K as 28.0, calculation shows that the measured ionizations at the irradiated surfaces due to the X-rays are 97 percent for Cu K and 89 percent for Cr K. Introducing this absorption correction and transferring to standard air conditions, the measured air ionizations of the beams used in the experiments are expressed as the saturation currents due to the ions produced in a cube 1 cm on its edge.

Except for irradiation, all males in any one experiment were treated alike. The control and irradiated males were mated singly to females having the composition scute, Bar, small wing, vermilion, tan ($= s_c\ B\ s_m\ v\ t$) in one sex chromosome and white miniature Beadex ($= w\ m\ B_x$) in the other. The scute Bar-chromosome prevents most of the crossing over and carries a recessive lethal effect. Complete counts of the progeny were made for these matings over a period of 8 days for copper and 10 days for chromium. (The breeding technique for the males irradiated with copper was somewhat different from that of the males irradiated with chromium. A copper irradiated male was bred to a single female, the pair being left in the culture bottle 8 days and then transferred to a second bottle for another period of 8 days. The average count of the progeny in the first bottle was determined and utilized as the number of surviving sperm for each dose. A study of the two bottles showed that the relative result was essentially the same but that the numbers of individuals found in the second bottle were much reduced as compared with the progeny from the first. In view of these facts, it was decided to leave the chromium irradiated pairs in the bottles for the full ten days and to omit the second bottle test. The differences in the absolute numbers of progeny for the survival curves of the copper and chromium series are accounted for on this basis.) After hatching commenced the Bar females were mated singly to $w\ m\ B_x$ stock

males. The progeny of these matings were then examined for recessive sex-linked lethals or visible mutations which might have been produced. If mutations were discovered further breeding experiments were made to locate the position of these changes within the chromosomes. The general scheme of the matings is the familiar *ClB* type as shown in figure 1.

Four types of information are determined from these matings:

1. The first noticeable effect of X-ray treatment of any organism is the sterility of this organism when it is bred to those of normal fertility. This sterility in the case of males is attributable to some alteration pro-

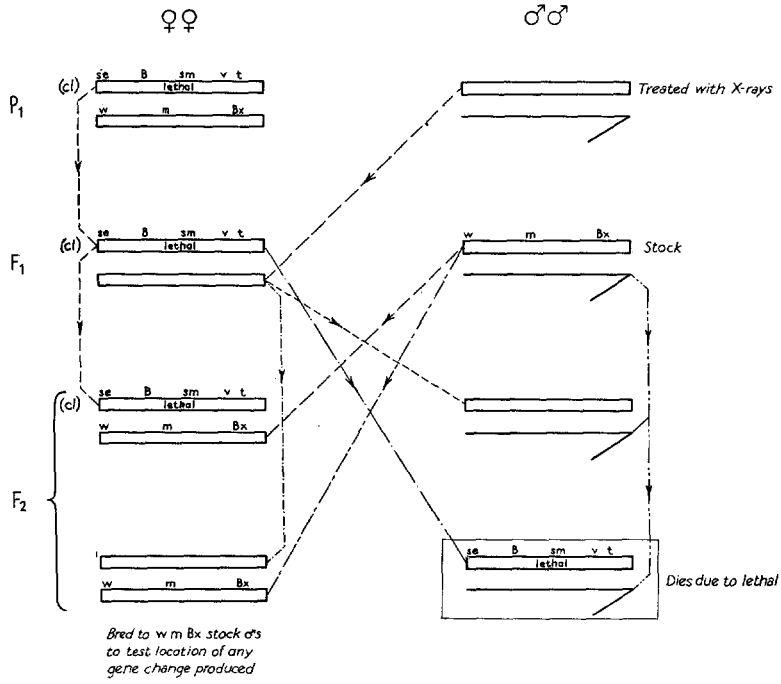

FIGURE 1.

duced within the sperm by the treatment. When such altered sperm fertilize normal eggs the embryos from these eggs die without becoming adult. The gene changes of this type produced by the X-rays may be looked upon as dominant lethals. These dominant lethals lower the number of adults surviving from matings sired by X-rayed males as contrasted with those sired by untreated males.

2. If one of these dominant lethals happens to be produced within the sex chromosome the lethal not only lowers the survival rate in this culture but it also lowers the number of females in relation to the number of males, since only females normally receive an X-rayed sex chromosome. This alteration in the sex ratio is consequently another direct measure of the number of dominant lethals produced by the X-rays.

3. The third class of data consists of the recessive lethals which are produced in the sex chromosome. These lethals manifest themselves by the fact that any F_1 female carrying such a lethal will have only female progeny. As the females containing the lethal do not die they may be bred for further testing the gene mutation and for locating its position within the chromosome.

4. A fourth group of information was obtained from the numbers of matings exhibiting visible mutations. These cases were analyzed for dominant or recessive sex-linked mutants and dominant autosomal mutations.

Data were collected on these four types of information for 8 durations of X-ray and for the two classes of rays, copper and chromium.

METHODS OF ANALYSIS

The survival curves measuring the dominant lethal mutations produced by the X-rays for the different dosages and wave lengths are presented in table 1.

TABLE 1

Survival curves for Drosophila sperm tabulated against time of exposure to X-ray and total X-ray exposure.

TIME IN SECONDS	COPPER SURVIVAL		SEX RATIO	TIME IN SECONDS	CHROMIUM SURVIVAL		SEX RATIO	e.s.u.
	NUMBER	RATIO			NUMBER	RATIO		
0	107.6±10.2	1.000	1.000±0.017	0	135.2±11.6	1.000	1.000±0.037	0
30	46.4± 8.7	0.431	0.960±0.029	37	69.0± 6.7	0.511	0.965±0.029	4460
60	18.9± 2.9	0.175	0.938±0.036	72	44.7± 3.0	0.330	0.909±0.027	8920
90	13.5± 2.9	0.126	0.867±0.042	111	17.2± 1.6	0.127	0.807±0.039	13380
120	7.4± 1.3	0.069	0.927±0.052	148	5.3± 0.6	0.039	0.792±0.064	17840
150	..	..	..	185	4.8± 1.1	0.036	0.934±0.081	22300
180	1.4± 0.3	0.013	0.779±0.067	222	1.3± 0.3	0.009	0.752±0.100	26760
210	.6± 0.1	0.005	0.898±0.108	259	0.9± 0.1	0.006	0.685±0.090	31220
240	.4± 0.1	0.004	0.654±0.157	..	..	..	..	35680
Ionization per sec./cm² in 1 cm of air			148.7				120.6	

The survival ratios of this table when plotted on semi-logarithmic scale have certain outstanding characteristics. The raw data form a straight line of constant slope without any lag period. If we think of the X-rays as a stream of bullets shooting at random at a target, this type of curve would be generated by the condition that one hit on the vital spot of the target would kill. This vital spot may be in one piece or may be subdivided into many parts scattered throughout the target, any one of which may be hit, the total area of these smaller spots equaling the entire area of the vital spot.

Sperm may be regarded as targets composed of compact bundles of

chromatin within which the genes are embedded, one gene following another as beads do on a string. Some of these genes are vital genes in the sense that alterations in their structure produce lethals which eventually cause the death of the organism. The death resulting from such changes probably occurs in Drosophila in the diploid development since it was possible to show that (1) females bred to X-rayed males contained sperm in numbers comparable to those bred to untreated males, (2) hatched eggs sired by X-rayed males died at a greater rate than those sired by normal parents, and (3) sperm and eggs carrying recessive lethal genes are not perceptibly decreased in numbers.

The vital genes which X-rays change to lethal factors are distributed approximately at random within the functional chromatin as shown by these experiments. By functional chromatin is meant chromatin carrying genes, as differentiated from that of the Y chromosome and the right-hand end of the X chromosome which, up to the present, and in spite of extensive work on Drosophila, has not been shown to carry genes in a manner comparable with the other chromatin material.

The vital genes are of two classes, those which produce their effect in haploid condition, or to make alternative classes, dominant vital genes *versus* recessive lethal genes, and those which cause the characteristic reaction only in diploid, recessive vital genes *versus* dominant lethal genes. The deaths brought about by the X-rays as shown in table 1 may be regarded as due to the rays hitting one of these vital spots with the consequent production of dominant lethal genes.

When an X-ray strikes matter it may be absorbed or it may pass through without effect. If the ray is absorbed it gives up one or more of its quanta with the coincident production of high speed electrons. These electrons in turn give rise to secondary electrons. The volume wherein these changes take place is small. This fact, together with the random distribution of the quantum absorptions over the chromatin, presents the chance element necessary to account for the experimental results.

The mathematical analysis of the set of conditions outlined above has been presented by several investigators (CROWTHER 1926). It leads to the equation

$$\text{survival} = \frac{\begin{matrix}\text{observed survival}\\ \text{after treatment}\end{matrix}}{\begin{matrix}\text{initial survival}\\ \text{without treatment}\end{matrix}} = e^{-ant}$$

where a is the probability of one shot hitting one of these vital spots, n is the number of shots in unit time, and t is the time of exposure. The consequences of this process may be visualized from a typical calculation.

In our experiment with copper radiation 148.7 electrostatic units were given per sec./cm^2 in 1 cm of air.

The number of ion pairs per sec. per cm^3 at the position of the irradiated sperm is consequently 148.7$\times$electrons in one electrostatic unit or 0.21 $\times 10^{10}$ ion pairs/sec. $= 3.12 \times 10^{11}$ ion pairs. sec./cm.^{3}

For material composed of atoms of low atomic weight these absorption coefficients vary directly with the density of the substance. If this conclusion is applied to chromatin, an assumption which cannot be far wrong, the amount of absorption per sperm head is $\frac{3.12 \times 10^{11} \times 1 \times 10^{-12} \times 1.00}{1.00 \times 1.165 \times 10^{-3}}$ $= 267$ ion pairs/sec./sperm. Since direct measurement of the dimensions of the sperm head are 7.36×10^{-4} cm long and 0.368×10^{-4} cm wide, hence—considering the sperm head a rectangular block of chromatin—the volume is 1.0×10^{-12} cm^3. The density of chromatin is assumed to be 1.00 and the density of the air at the temperature used is 1.165×10^{-3}.

In order to obtain the number of X-ray quanta absorbed per second it is necessary to know how many ions are liberated by one quantum absorbed in air. The best available measurements (KULENKAMPFF 1926) show that X-rays of the quality used require about 35 volts for each electron pair. The voltage equivalent of the K$-\alpha$ lines of copper follows from the quantum relation Voltage (in K.V.) $= 12.34$/wave length of copper X-rays (1.537) $= 8.029$ K.V.

The number of ion pairs arising through the absorption of one quantum of Cu K$-\alpha$ X-rays is $\frac{8{,}029}{35} = 229$. The average number of quantum absorptions per second is $\frac{267}{229} = 1.163$.

The average number of absorptions which actually produced death was determined from the experimental data by fitting the curve previously derived, survival $= e^{-ant}$, to the actual data by the method of least squares. The resulting curve was $Y = e^{-0.024t}$.

If the sperm were entirely composed of vital recessive genes then every hit would be expected to kill, or the rate of killing with time should be 1.163 per second. The exponent 0.024 per second for the actual data shows that this is not the case. The chance of hitting this vital volume within the sperm clearly decreases as its volume relative to the whole sperm becomes less. The theoretical absorption 1.163 per second represents the whole volume. The effective absorption 0.024 represents the vital volume. Their ratio, 1:0.021, represents the whole volume compared with this vital volume. In other words, the normal sperm area has 21 thousandths of its volume composed of vital recessive genes.

In these calculations and those which follow, extensive use is made of the data on the size of the sperm and the chromosomes composing them. These measurements were obtained from material fixed in modified Bouin and stained with iron haematoxylin. Suitable sperm which were separate and distinct from each other were photographed at 770 diameters. The photographs were then made up on lantern slides and projected on a screen. The sperm were drawn from the projected images and the magnitude of the enlargement determined. The average size of the sperm head was 7.36×10^{-4} cm in length and 0.368×10^{-4} cm in breadth. The size of the individual chromosomes within the sperm head was determined from the oögonial divisions. The technic of fixation staining and enlargement was the same as that described for the sperm save that the initial magnification of the photograph was 2500 diameters. One of the division figures utilized contained besides the normal female complex a Y chromosome. The measurements of the different chromosomes are:

Sex or X chromosome	Length 1.56×10^{-4}cm	Average breadth 0.34×10^{-4}cm	Area 5.27×10^{-9}cm^2
II chromosome	Length 2.21×10^{-4}cm	Average breadth 0.33×10^{-4}cm	Area 7.22×10^{-9}cm^2
III chromosome	Length 2.80×10^{-4}cm	Average breadth 0.34×10^{-4}cm	Area 9.71×10^{-9}cm^2
IV chromosome	Length 0.28×10^{-4}cm	Average breadth 0.26×10^{-4}cm	Area 0.58×10^{-9}cm^2
Y chromosome	Length 1.85×10^{-4}cm	Average breadth 0.30×10^{-4}cm	Area 5.61×10^{-9}cm^2
Total haploid size with X chromosome	Length 6.85×10^{-4}cm	Average breadth 0.33×10^{-4}cm	Area 22.78×10^{-9}cm^2
Total haploid size with Y chromosome	Length 7.14×10^{-4}cm	Average breadth 0.32×10^{-4}cm	Area 23.12×10^{-9}cm^2

Figure 2 shows a sample photograph of the sperm used in obtaining these measurements. Figure 3 shows one of the metaphase oögonial figures, containing a Y chromosome measured for the size of its chromosomes.

While somewhat aside from the problem under discussion, the comparison of the measurements of the sperm head with those of the condensed metaphase chromosomes is significant from the viewpoint of the mechanism by which these chromosomes may be condensed into the sperm head. The maximum breadth of the sperm head represents the maximum breadth which a chromosome assumes if the sperm is to be one chromosome wide. This breadth is 0.368×10^{-4} cm or somewhat smaller. The breadth of each of the chromosomes is approximately the same as this figure, 0.33×10^{-4} cm. If chromosomes reach the stage of extreme condensation at the metaphase, then these results show that they must lie end to end in the sperm head. The total length of the chromosomes under these conditions should be the same as the length of the chromatin in the sperm head. The length of the sperm head is 7.36×10^{-4} cm, although this length is again probably somewhat large. The total length of the haploid chromosomes is 6.85×10^{-4} with the X, or 7.14×10^{-4} cm with the Y.

The result therefore corresponds with the view that the chromosomes lie end to end in the Drosophila sperm. These measurements on fixed tissue, while somewhat less than they would be if taken from living tissue are of the same order of magnitude.

The sex ratios in the second group of data were determined from the relation ♀/2♂ since the presence of the lethal in the *ClB* test females reduces the males to half their true number. These sex ratios were then

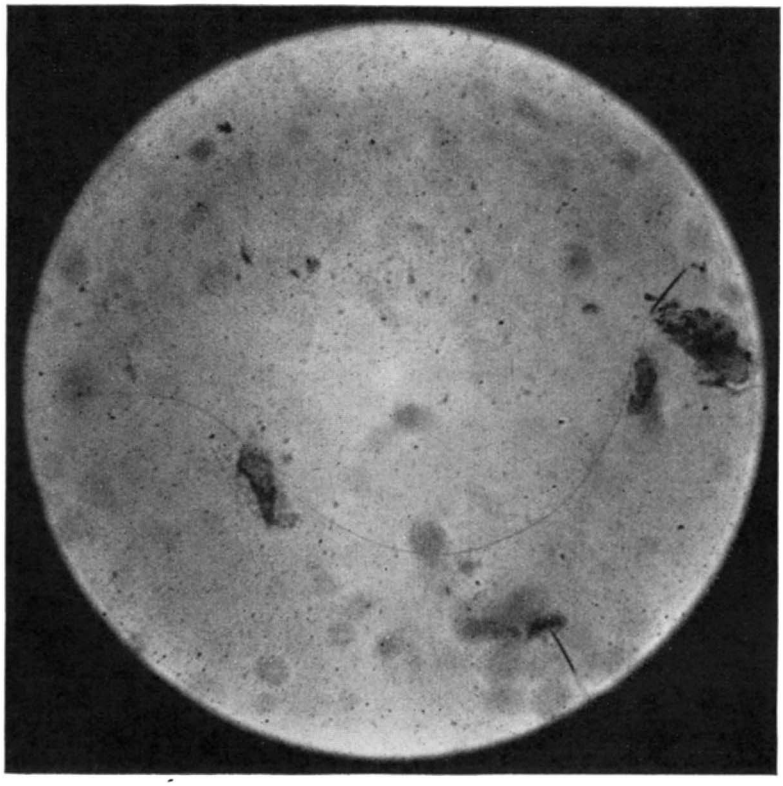

FIGURE 2.—Drosophila sperm taken from female. The tail of this sperm is at least three times as long as shown, and quite possibly longer since it may have been broken in handling the material. The acrosome, cytoplasmic sheath, etc. of the sperm head is stained in this preparation. Magnification 770.

adjusted to 100 as the ratio for the untreated class. The analyses of the sex ratio data were entirely similar to those for the survival curves of the whole organism as given above.

The recessive lethals could be tested and placed in their relative positions within the chromosome. The presence of one recessive lethal in general had no influence on the presence of another. It was therefore possible to determine whether or not a chromosome had more than one

lethal. This fact makes it possible to treat the data in two ways, leading to the same general conclusion.

If a is the chance of one shot hitting the target and n the number of

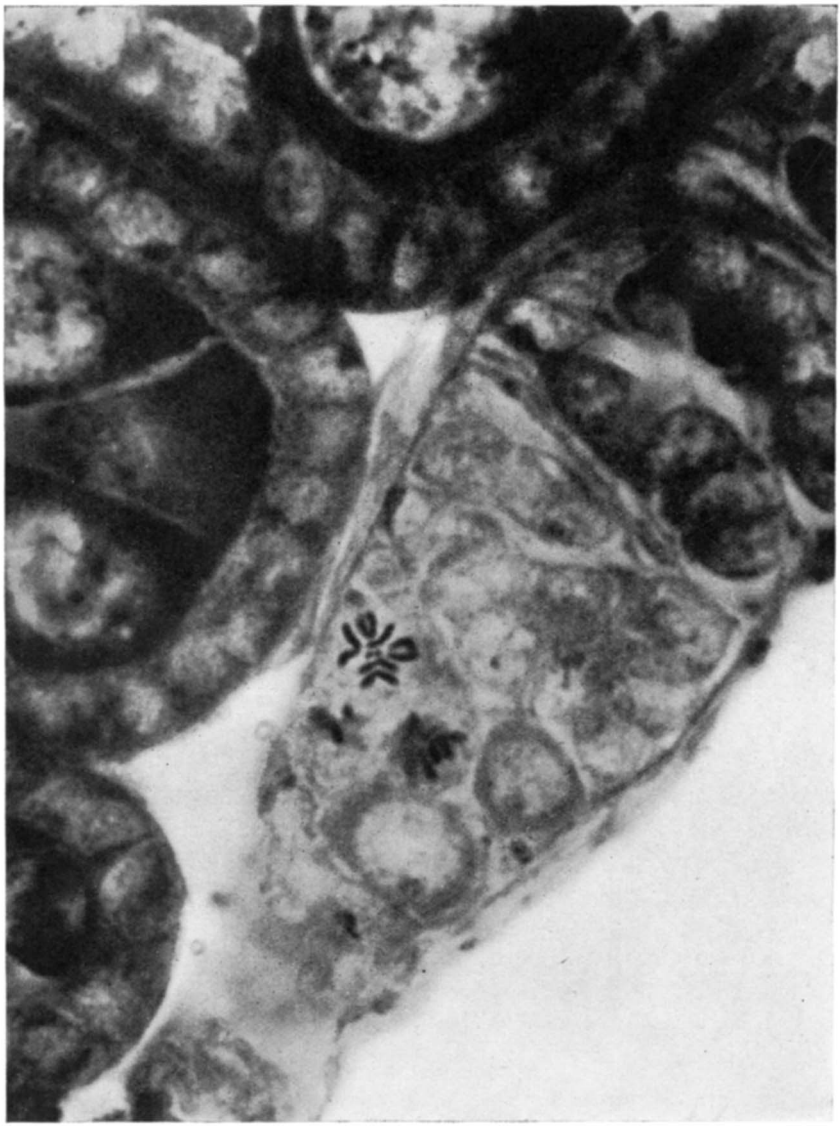

FIGURE 3.—Drosophila chromosome plate showing diploid group plus an extra Y. Magnification 2500.

shots in unit time, then the chance in a given number of units is $Y = ant$ instead of e^{-ant} as is the case when one hit makes it impossible to distinguish another hit on the target. The rate of change is again an however.

This curve is a summation of hits, chromosomes having two recessive lethals being counted twice, those with three being counted three times, etc. Obviously this method has real difficulties when it comes to determining the presence of two or more lethals closely associated in the chromosome.

The data may also be treated exactly the same as those for the dominant lethals to obviate this difficulty. The equation for the chromosomes which show no recessive mutations, or what might be looked upon as the survivors, becomes

$$Y = \frac{\begin{array}{l}\text{observed chromosomes}\\ \text{lacking recessive lethals}\\ \text{after treatment}\end{array}}{\begin{array}{l}\text{initial chromosomes lacking}\\ \text{recessive lethals before}\\ \text{treatment}\end{array}} = e^{-ant}$$

This curve is the curve which HANSON and HEYS (1929) should have applied to their data since their technique shows only the proportion of chromosomes which had no lethal mutations and not the total number of lethal mutations per given number of chromosomes. The analysis of their beautifully regular data supports this view as the curve e^{-ant} fitted to the proportion of the chromosomes which escape being hit fits the material more accurately than the curve $Y = ant$ worked out from the data as they present them.

EXPERIMENTAL DATA

Table 1 presents the data on the survival rates of sperm irradiated with increasing amounts of either copper or chromium X-ray. These data are plotted in figures 4 and 5. The middle bar for each irradiation gives the survival value attained in the experiment. The length of the heavy line shows the probable error of this determination. The data are plotted on semi-logarithmic scale since such a plot presents the curve $Y = e^{-ant}$ as a straight line. The equations for the survival curves for the copper irradiation are $Y = e^{-0.0244t}$ or $Y = e^{-0.0_3164e.s.u.}$ depending whether time of exposure in seconds or electrostatic units of irradiation are used as the unit of measure.

The survival curves for the sperm exposed to irradiation from chromium are $Y = e^{-0.0198t}$ or $Y = e^{-0.0_3164e.s.u.}$.

It is obvious that the curves of survival for the two treatments are the same when the electrostatic unit is used to measure the X-ray. The difference in the time curves is due to the fact that copper irradiation gives the same number of electrostatic units in less time than chromium. The straight lines of figures 4 and 5 are the theoretical curves plotted from the electrostatic unit equations.

The data on the ratios of females to males after irradiation are likewise presented in table 1. These data measure the survival value of the sex or

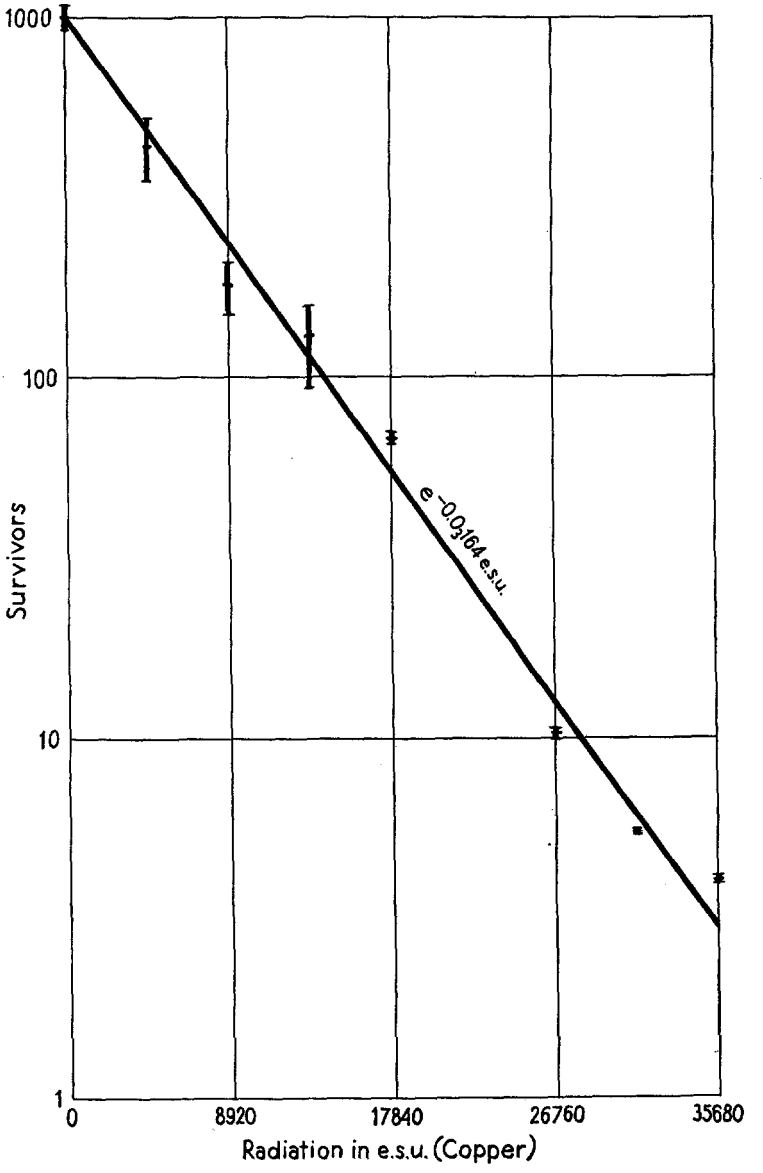

FIGURE 4.—Survival of Drosophila sperm irradiated with X-rays from a copper target.

X chromosome as separated from the other chromosomes, autosomes. Figures 6 and 7 present the curves for these two sex ratios plotted on semi-logarithmic scale. The cross bar shows the result of the particular treat-

ment and the heavy vertical line the probable error of this determination. There is more variation in these data than there was in those for the survival curve as the larger quantities of irradiation reduce the survivors

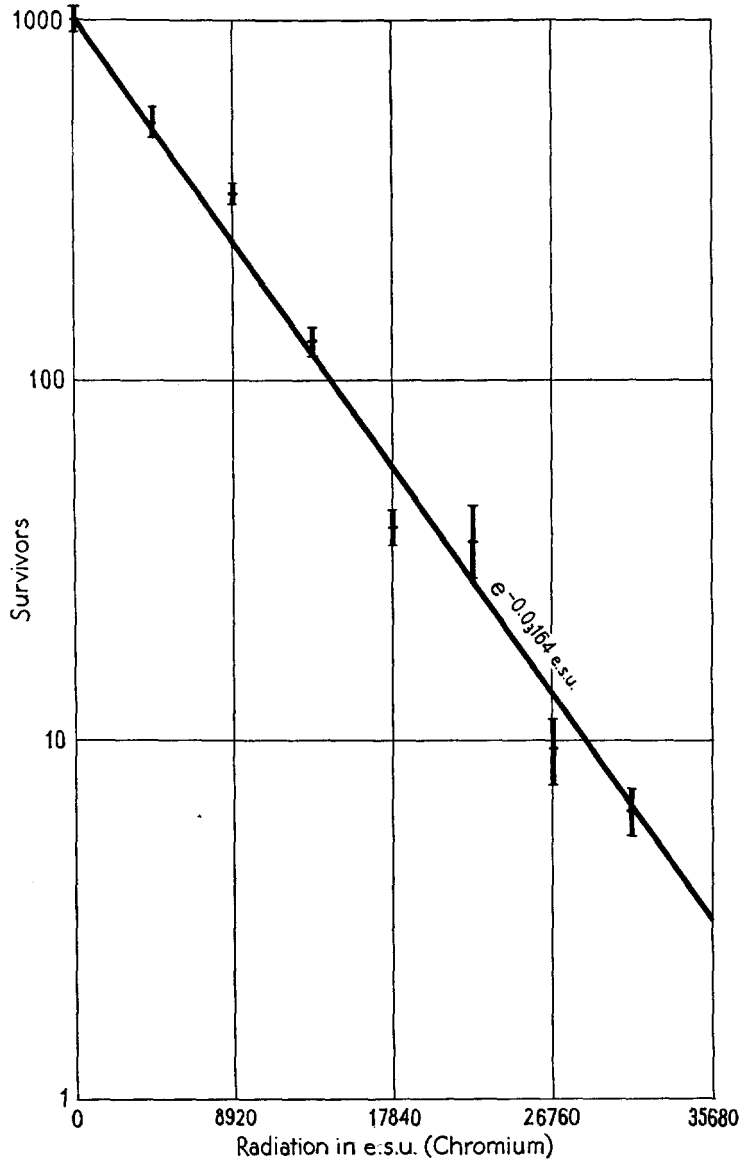

FIGURE 5.—Survival of Drosophila sperm irradiated with X-rays from a chromium target.

and consequently increase the observed variation. The data, however, clearly point to the linear nature of the semi-logarithmic plot. The equations for the changes of the sex ratios with increasing amounts of exposure

to the X-rays were for copper $Y = e^{-0.00114t}$ or $Y = e^{-0.0_5766e.s.u.}$. The sex ratios of the flies surviving the chromium irradiation had the equations

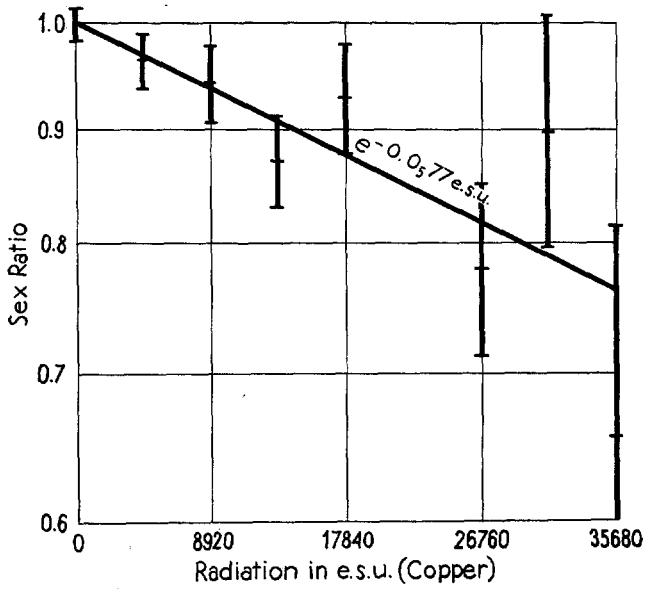

FIGURE 6.—Sex ratio of surviving Drosophila sperm after irradiation with X-rays from a copper target tube.

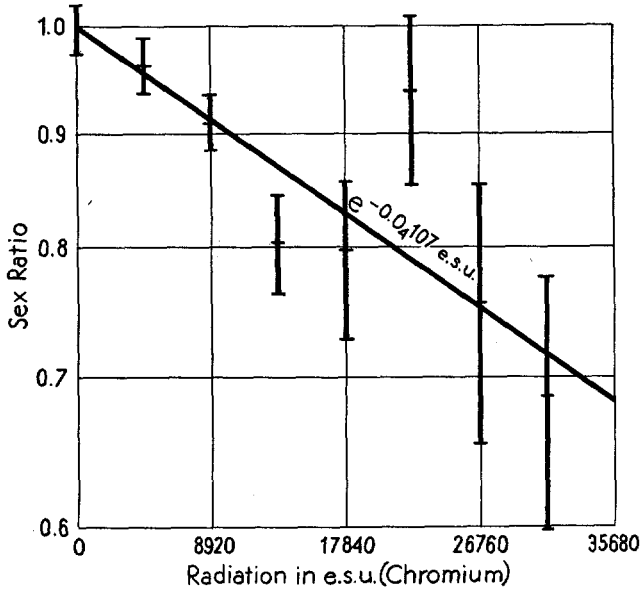

FIGURE 7.—Sex ratio of surviving Drosophila sperm after irradiation with X-rays from a chromium target tube.

$Y = e^{-0.00130t}$ or $Y = e^{-0.0_4107e.s.u.}$ The straight lines of figures 6 and 7 represent these equations.

It will be noted that the depression in sex ratio due to copper irradiation is different from that due to chromium. This difference is not significant since it has a standard error slightly more than itself ($P = 0.35$).

The recessive lethal mutations furnish data which cannot be collected from the dominant lethals, as this type of mutation does not kill the fly except when homozygous. This makes it possible to separate these lethals into pure lines, locate them within the chromatin matrix, etc. These mutations appear at random in relation to each other and to the visible mutations. If they appear at random as compared with the dominant lethals then the deaths brought about by the dominant lethals do not alter the form of the curve for the rate at which the recessive lethal mutations are produced. The assumption is borne out by the data.

Were it possible to count all of the recessive lethals produced, chromosomes in which only one lethal was produced, chromosomes with two lethals, three lethals, etc., then the form of the curve, representing percentage of lethals produced against electrostatic units of irradiation to which they were exposed, would be a curve of the form $Y = ant$.

There is a grave technical difficulty in determining the presence of double lethals in a chromosome when these lethals are close together. It is possible however to arrive at these same constants from the curve showing the percentage of chromosomes in which no lethal is produced. This curve is entirely like the survivor curve of the dominant lethals, the ant having the same meaning as in the curve above $Y = e^{-ant}$.

Table 2 gives the data for the percentage of sex-linked recessive lethals, including the doubles, as they were experimentally obtained. Beside this column is given the percentage of chromosomes tested which contained no recessive lethals. The percentages of visible sex-linked factors and dominant autosomal factors are given in columns 4, 5, and 6 for the copper irradiations and 10, 11, and 12 for the chromium irradiations.

The data of table 2 lead to the equations $Y = e^{-0.0_2165t}$ or $Y = e^{-0.04112e.s.u.}$ to describe the rate of recessive lethal production for sperm exposed to copper irradiation. The rates of lethal mutation for the sperm exposed to irradiation from chromium have the equations $Y = e^{-0.0_2162t}$ or $Y = e^{-0.04135e.s.u.}$ These data are plotted in figures 8 and 9.

A comparison of figures 8 and 9 shows that the rate of lethal mutation for sperm exposed to copper irradiation is somewhat less than that from chromium irradiation. The difference in the two rates is not significant, however, since the standard error of the difference of the two rates is 0.0_664 and the probability that the two distributions are alike is 0.15. The curves for the percentage of mutations with time of irradiation by X-rays showed that these gene changes are produced at a constant rate irrespective of wave lengths. If percentage of mutation is plotted against energy

TABLE 2

Relation between the X-ray dose and the ratio of recessive lethal or visible mutations.

TIME IN SECONDS	COPPER SEX-LINKED MUTATIONS RECESSIVE LETHALS	CHROMOSOMES NOT CONTAINING RECESSIVE LETHALS	RECESSIVE VISIBLE	AUTOSOMAL DOMINANTS TESTED	AUTOSOMAL DOMINANTS UNTESTED
0	0.000±	1.000±	0.000	0.0000	0.0008
30	0.043±0.006	0.960±0.005	0.0054	0.0024	0.0062
60	0.089±0.013	0.911±0.013	0.0097	0.0008	0.0188
90	0.117±0.015	0.888±0.016	0.0152	0.0120	0.0142
120	0.200±0.021	0.813±0.021	0.0375	0.0066	0.0198
150	..	..	..	..	..
180	0.292±0.038	0.708±0.038	0.0152	0.0234	0.0132
210	0.350±0.051	0.675±0.050	..	0.0158	0.0158
240	No progeny	..	..	0.0303	..

TIME IN SECONDS	CHROMIUM SEX-LINKED MUTATIONS RECESSIVE LETHALS	CHROMOSOMES NOT CONTAINING RECESSIVE LETHALS	RECESSIVE VISIBLE	AUTOSOMAL DOMINANTS TESTED	AUTOSOMAL DOMINANTS UNTESTED	e.s.u.
0	0.006±0.004	0.994±0.003	0.000	0.0000	0.0030	0
37	0.068±0.010	0.935±0.010	0.0036	0.0025	0.0045	4460
72	0.124±0.011	0.878±0.011	0.0113	0.0048	0.0114	8920
111	0.193±0.017	0.815±0.017	0.0164	0.0106	0.0169	13380
148	0.265±0.036	0.779±0.034	0.0147	0.0130	0.0585	17840
185	0.188±0.029	0.825±0.029	..	0.0036	0.0216	22300
222	0.318±0.067	0.682±0.090	0.0454	0.0093	0.0278	26760
259	0.348±0.067	0.652±0.067	0.0435	0.0256	0.0342	31220
						35680

of the X-rays as the other variable, the curve shows that for a given quantity of energy the gene changes produced are constant. The wave lengths used in these experiments were rather long. HANSON and HEYS have used the gamma and beta rays of radium on Drosophila. In their experiments the rate of mutation, as measured by recessive lethals, increased directly with the dosage. In their first series of experiments we are unable to determine these dosages in electrostatic units. However, if we assign to one of their points a like value to ours in mutation rate, and dosage, we find that the two series of observations correspond. In another series of experiments they give a dosage of 6316 e.s.u. to their flies and obtain a mutation rate of 4.7 percent. This rate is somewhat lower than that obtained in our experiments. MULLER gives his tungsten X-ray irradiations for two points in e.s.u. units together with the rates of mutations. OLIVER gives like data which were taken in the same laboratory. For the e.s.u. readings these rates of mutation are considerably larger than those of either HANSON and HEYS or the writers. The fact that the radium

radiation produces essentially the same effects as the long waves of chromium and copper certainly suggests that wave lengths within these ranges are not important to the changes which X-rays produce in tissues.

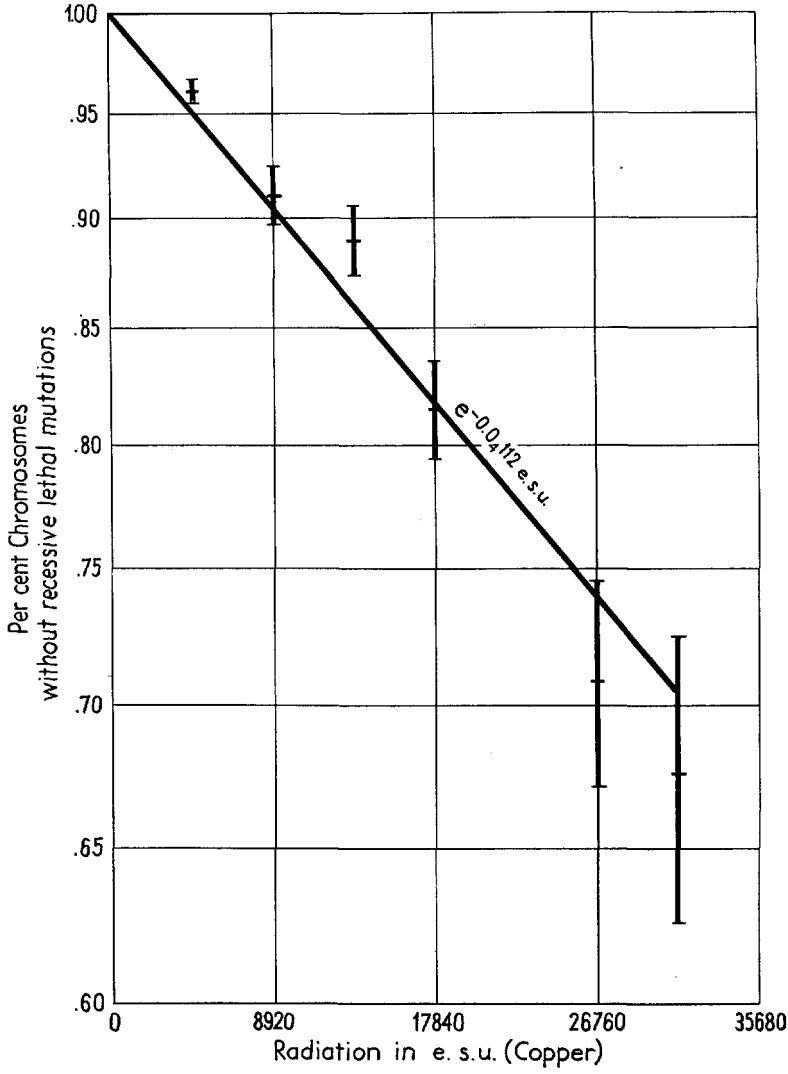

FIGURE 8.—Relation between the rate of recessive lethal mutation and exposure to X-ray irradiation from copper target.

It will be recognized that these diverse types of irradiation have produced their effects directly on the sperm without the intervention of crossing over. In this sense they would support STURTEVANT'S view that crossing over must be discounted as a probable explanation of so-called natural mutation.

The visible sex-linked recessive genes which were produced by the X-ray treatments cover a rather wide category of characters. The trends of the frequency of these mutations with increasing dosages of X-ray are

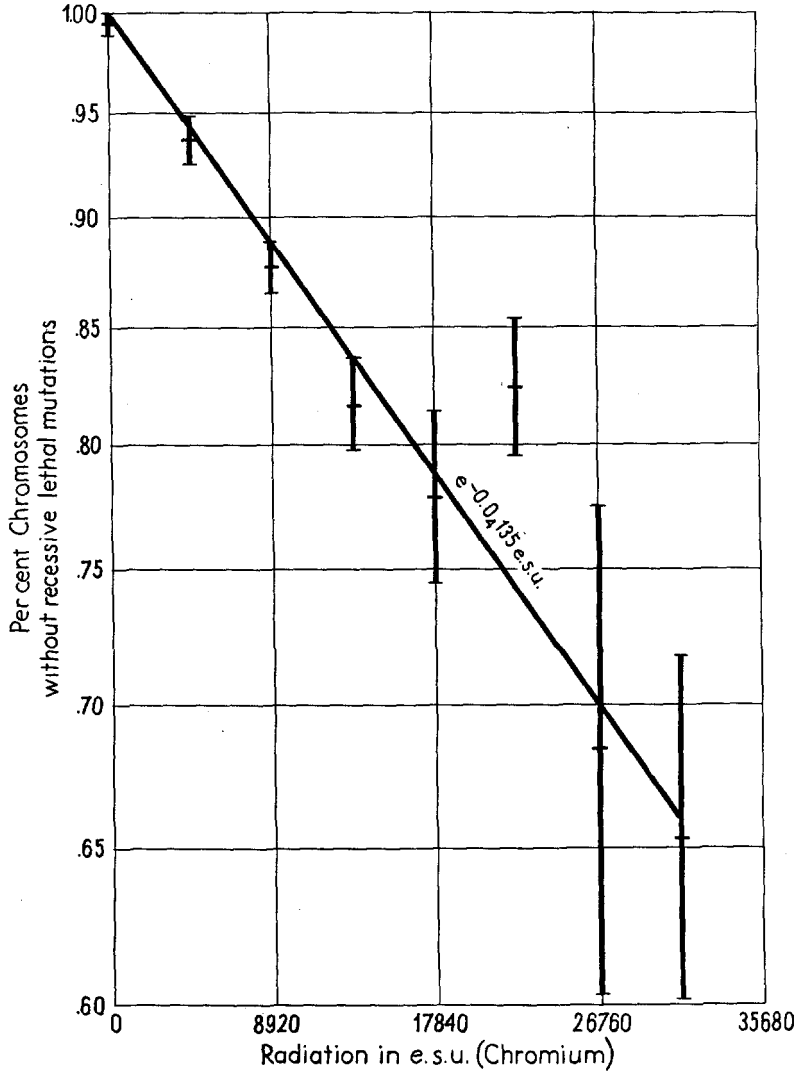

FIGURE 9.—Relation between the rate of recessive lethal mutation and exposure to X-ray irradiation from a chromium target.

given in table 2. This trend is quite irregular due to the relatively small numbers of these mutations produced. The rate of mutation rises with increase in the dosage of X-ray, however. A comparison of the rates of these mutations for the two types of X-ray leads to the conclusion that they are essentially the same. The number of visible mutations is about

one-tenth that of the recessive lethal mutations. In fact, for all data, there were 44 fairly clear and apparent visible mutations produced in the same group of sperm which produced 320 recessive sex-linked lethal mutations or 13.7 visible sex-linked mutations to 100 which were recessive sex-linked lethals.

The dominant, visible mutations in the autosomes, were divided into two groups. Each one of the first group was tested for location within its chromosome. Those of the second group failed to breed and were classified as untested. Here again it will be noted that these mutations increase with increasing X-ray dosage. The general trend of these curves is similar for both treatments; their irregularity is no doubt due to the small numbers of these mutations which were produced. The rate at which the dominant visible autosomal factors were produced is comparable with that of the recessive sex-linked visible factors. However, as the autosomes consist of about 10 times as much gene-containing chromatin as the sex chromosome, it is apparent that the dominant visible loci in the autosomes are more widely scattered than the loci for the visible sex-linked genes.

DISTRIBUTION OF THE GENE CHANGES

Three hundred and fifty-six mutated genes were tested for their loci within the sex chromosome. These loci were distributed over the sex chromosome's entire genetic length. The frequency distribution of the mutated genes was plotted on the conventional sex chromosome divided into units of 10, each series separately. The comparison of these distributions for the seven classes of chromosome length 0–10, 10–20, 20–30, 30–40, 40–50, 50–60, 60–70, showed a χ^2 of 10.1 for the six degrees of freedom. Because it is entirely probable ($P = 0.13$) that the two distributions are the same, they have been combined. Figure 10 shows these combined frequencies plotted as percent of the total mutations. The class interval for the first and second groups is 5 units of chromosome length. Each of the other intervals represents 10 units of the genetic chromosome.

Figure 10 shows much the same distribution of the mutated genes within the sex chromosome as found for the so-called natural mutation tabulated by MORGAN, BRIDGES and STURTEVANT, or for those produced by the heterogeneous irradiation of tungsten as tabulated by MULLER. (It is necessary to estimate the frequencies of MULLER'S tungsten mutations from the graph, a method which, while approximately accurate, is not exact.) In common with these investigations there is an apparent close crowding of the mutation at the 0 end of the sex chromosome. This crowded area is followed by an area showing fewer gene loci. This depressed area is in turn followed by areas with mounting frequency of mutation to another crest at 50 to 60 units. From this point the number of the

genes per unit of genetic chromosome decrease to zero, at slightly beyond the 70 unit mark. One noticeable difference between our data and those cited is that no depression is noted in the region 40 to 50 units.

It seemed worth while to determine whether these three different groups of data could reasonably come from the same population. The comparison of the data of MORGAN, BRIDGES and STURTEVANT with this material showed a probability for their coming from the same population of 0.05. Comparison with the data of MULLER (1928) showed a similar probability, 0.02. The probabilities are on the borderline of significance. In the comparison over half the contribution to the χ^2 came in the first class, for on the basis of these other data, our relative frequency of factors in this

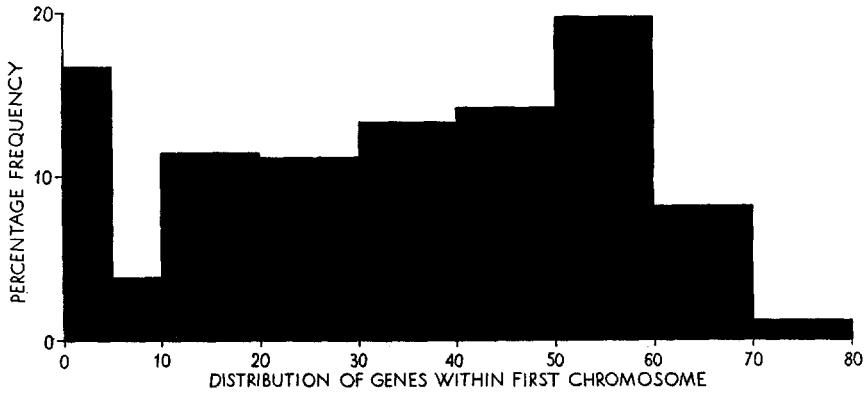

FIGURE 10.—Gene mutations as they were distributed within the genetic sex chromosome.

region is low. Another third of the contribution to χ^2 comes from the region 40–50 genetic units where we find a higher frequency of genes than do the other investigators. These differences may be significant. Since, however, the probabilities are in one case within three times the probable error and in the other but slightly beyond it, it seems likely that the differences in the three distributions are rather to be attributed to chance.

The mutant genes have been classified as lethals and visibles. These two classes intergrade and for that reason are more or less arbitrary. Figure 11 will enable the reader to appreciate the effects of these mutations on the vital processes of the organism. The data for the copper and chromium irradiations have been combined since comparison has shown them to be random samples of the same population.

Figure 11 shows that about 75 percent of this sample of genes are lethal. This means that the normal allelomorphs of 75 percent of this type of genes are vital to the animal's well-being. Seven percent show lesions which are severe enough to kill all but 1 to 5 percent of the progeny. Seven percent do not affect vital processes sufficiently to influence the organism's

viability to a marked degree. These results are comparable to those of MULLER on tungsten irradiation and of MULLER and ALTENBURG on natural mutations.

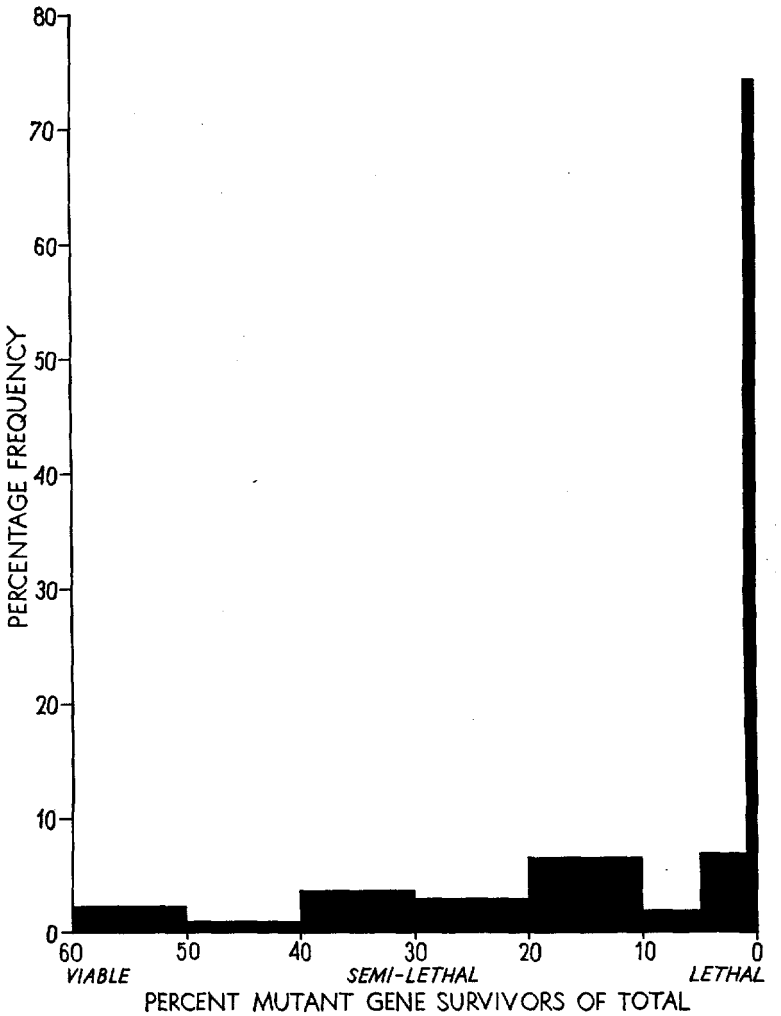

FIGURE 11.—Frequency distribution showing the sample of genes and the proportions of them which are completely lethal, produce pathogenic effects making them semi-lethal, and are fully viable. The ordinates represent percentage of genes in given class of the total. The abscissa gives the percentage of survivors in the gene mutant class divided by the whole number of survivors. 0=completely lethal; 50=normal viability.

Crossing over effects are noted with several of these factors. Aside from those which proved to be translocations between chromosomes, about 20 percent of the factors depressed the crossing over between w and B_x to about half its normal value.

The exact comparison of the visible genes one with another and with those found elsewhere is of particular significance to the argument which is to follow. For this purpose we shall consider only the factors which had fair viability, defined as 20 percent or better survival, classifying the rest as lethals. With this viability it seems reasonably certain that if any one of these factors had reappeared it would have been recognized. Our experiments produced 44 such factors, and 42 factors which, although generally lethal, occasionally produced a few living mutants. (These last 42 were classified as lethals, since they might have been completely lethal had they reappeared elsewhere. In all cases these 42 mutant forms, when they did survive, showed pronounced external abnormalities, no doubt but an indication of much more extreme changes within the internal organs.)

Of the 44 gene changes, which if duplicated should be discovered, 5 were proven to be allelomorphic to genes described by MORGAN, BRIDGES and STURTEVANT. These 5 loci were yellow (some differences from the previous yellow gene), echinus twice (the second echinus also showing differences), ruby once, miniature and forked. Besides these proven allelomorphic genes there were 6 others which were probably identical with others previously described. These genes were chlorotic, facet, singed (is ♂ sterile), ascutex twice (one more extreme than the other), furrowed twice (one more extreme than the other), and fused. The criteria for the conclusion that these genes were like those described are a similar morphology and location within the chromosome.

These data allow us to form an idea of the number of these genes within the sex chromosome. As a first approximation we shall assume that all of this class of genes are equally likely to mutate under the influence of X-rays and that when they do mutate they will be equally detectable. Two samplings have been made from this total population of loci. The first sampling followed the period of years during which the evidence for the existence of any of the loci came as the chance occurrence of natural mutations, thus giving rise to a Mendelizing difference. MORGAN, BRIDGES and STURTEVANT have tabulated and described these genes. The sex chromosome genes of this series, comparable to the 44 which we have described above, fall into 42 loci. If this same population of genes is sampled, the chance of obtaining a gene occupying any one of these loci in one draw is thus $42/\text{Total loci} \times 44$.

The experiment showed 6 proven identical loci and 8 very probable identical loci, or 14 in all. The experimental results set the answer to the above equation as 14; $42/\text{total loci} \times 44 = 14$ or total loci $= 132$. Thus the sex chromosome may contain 132 genes which can undergo rather easily detectable and fairly vital mutations. It may be argued that only the 6

proven allelomorphs should be used in the calculation. This would lead to an estimate of the number of loci as 308. We believe, however, that it is proper to consider 14 out of the 44 mutants as being allelomorphic to those previously known.

MULLER has published some data from which a similar estimate can be made. In 128 mutations of all kinds he obtained 20 which were at least 10 percent viable and conspicuous. Of these 20, 7 occupied loci previously known and tabulated among the 42 cited above; of these 7, 5 were proven allelomorphs. These data give 120 loci as the estimated number for this type of factors in the sex chromosome. Of the 7 loci found by MULLER in his tungsten X-ray work one, facet, was common to our series of 14.

The number of genes, 132, in the described category, must be regarded as a minimum, since if some of these genes mutate more readily than others we shall obtain a lower number than are actually present. Another independent estimate of the numbers of these sex-linked loci may be obtained from the ratio of the numbers of mutations noted once in the series to the numbers of those noted twice, three times, etc., after the manner developed by MULLER in his study of natural mutations. For our data 38 mutations appeared once or more; 6 appeared twice. If all of these loci are equally susceptible to the effects of the X-rays and if the genes within them change to produce equally detectable mutations then the ratio of the total number of these genes to the number that mutate once is equal to the ratio of the number that changes once to the number which changes twice, etc., or

$$\frac{N_{x+1}}{n_x} = r \text{ (a constant)}.$$

The series above gives the values of r as $6/38 = 0.158$. The value for the total number of the gene loci in this chromosome would consequently be 38 divided by 0.16 or 238, a somewhat larger figure than that obtained by the other method.

MULLER has applied this reasoning to the data published by MORGAN, BRIDGES and STURTEVANT for natural mutations. All chromosomes were used in this determination. A very real error is introduced here since examination of the data shows that sex-linked mutations are more easily detected at their original and repeated occurrences than are the autosomal factors. Furthermore calculations from these data relate essentially to the visible factors since any arrangement of this data would make it heavily overloaded with this class. Basing the calculations on the sex-linked visible factors, we find that 7 loci mutated 8 or more times; 8 loci 7 or more; 12 loci 6 or more; 14 loci 5 or more; 15 loci 4 or more; 18 loci 3 or more; 23 loci 2 or more and 42 loci 1 or more. This series leads to the values begin-

ning with the 8 appearing loci of 0.87—0.67—0.86—0.93—0.83—0.78 and —0.55. All values save the last are remarkably alike. The last value would seem to indicate either that genes under natural conditions have idiosyncrasies which make them characteristically high, intermediate, or low in their mutation rate, or that a mutation is more apt to be described once in this summary of genes than to appear in the list of repeated appearances, a purely spurious correlation. The latter might well happen in view of the tremendous difficulties involved in accumulating such data over a period of years. However that may be, the work of STADLER would argue for the first view. The consistency of the r values after that for genes appearing only once would argue against it. If there is heterogeneity in the rates between different genes then the number of genes, 76, obtained by using the $r = 0.55$, will certainly be too low.

MULLER has estimated the gene number by another technique. From ZELENY'S and STURTEVANT'S data on the frequency of crossing over of the Bar gene with itself he reaches a value of 0.2 of a genetic unit as the space occupied by this gene. STURTEVANT'S paper of 1925 in conjunction with his later paper of 1929 would seem to make this rate too high. If we calculate the percentages of crossing over, assigning to the data double the rate of the more viable class where necessary, and weight the 14 different types of matings by the square root of their number we arrive at the figure 0.0009 as the percentage interchange in the askew crossing over, somewhat less than a tenth of a genetic unit for the Bar gene. This reasoning would lead roughly to a total of 800 loci for the genetic sex chromosome. But as MULLER points out, there are drawbacks in this evidence; the crossover relationships are disturbed by the presence of the Bar gene, as shown by the asymmetry of crossing over, and by the fact that the Bar region of the chromosome shows a larger than normal proportion of loci when plotted against the genetic chromosome.

This discussion leads to a numerical value for the loci of sex-linked recessive genes which produce visible, easily distinguishable, characters between 75 and 268, with an average of 189. Provisionally, we shall adopt the value 175 to represent these gene loci.

The question arises as to whether the lethal genes as a class have the same mutability as the visible genes. There are several facts which suggest that they have. The apparent similarity in the distributions of these two types of genes over the chromosome as regards time and space, points to the conclusion that no one gene or even a few genes can be so susceptible to change that they account for the observed results. The conclusion that the lethal genes have the same mutability as the visible genes is borne out by another type of evidence. The reasoning may be illustrated by the chromium data where the exposure was 17800 e.s.u. The chance of obtain-

ing a recessive lethal gene change in any one sex chromosome is the number of these mutations divided by the total number of chromosomes tested, or 18 divided by 68=0.265. If these changes are made at random, there should be chromosomes which show two recessive lethal mutations. Such chromosomes have been found. Our technique undoubtedly does not obtain them all, however, since the difficulty of separating and proving double lethal mutations increases markedly as their loci approach each other. We shall therefore consider only those cases in which one gene occupied a position in the chromosome left of miniature and the second between miniature and Beadex. The problem may be viewed as follows. There are 36 genetic units of chromosome left of miniature and 24 units between miniature and Beadex. In our experiment 26.5 percent of mutations were obtained in 100 chromosomes. If we assume that the genetic units between the factors, yellow and miniature, and miniature and Beadex represent the proportion of potential recessive lethal factors, the ratio of the loci becomes 36 and 24. In random sampling from a bag containing balls to represent these loci there would be 36 red balls representing the loci left of miniature and 24 white balls representing the loci between miniature and Beadex. Besides these there are 167 yellow balls, a number sufficient to represent the draws when no lethal is obtained. The chance of drawing a lethal is

$$\frac{36+24}{36+24+167}=\frac{60}{227}\text{or } 26.5 \text{ percent}$$

as in the actual experiment. In any one draw the chance of obtaining a red ball is 36/227. In the second draw the chance of obtaining a second lethal in the mB_x region is the chance of drawing a white ball 24/227. The chance of drawing them both together is consequently $36/227 \times 24/227$.

But there is a second way in which this result may be accomplished since the white ball might be drawn first and the red one second. The chance of drawing a chromosome with two lethals each in the region indicated is $2(36/227 \times 24/227) = 0.0335$ or for the 68 draws of this experiment 2 chromosomes should be found with lethals in each of these regions. The experimental results confirm this hypothesis since we obtained 3 double lethals. Extending this process to the other irradiations the expectation of finding chromosomes with two lethals in these regions was, commencing with the initial irradiation, 4460 e.s.u., 0, 3, 3, 2, 1, 1, 1. The numbers actually obtained for the same arrangement were 1, 1, 2, 3, 1, 0, 0. The copper series is not quite in such good agreement, due partly we believe to the fact that it was the first experiment and that consequently our technique was not as good. The expectation for this series was 1, 1, 2, 3, 0, 3, 3. The observed results were 2, 0, 1, 2, 0, 0, 1. In both experiments fewer double lethals were found than expected, due partly we believe to our technique.

In spite of this difficulty the results are well within expectation since comparison of the observed with the expected leads to a P of 0.5. To this extent therefore these data support the view that recessive gene mutations are produced at random.

Testing lethal mutations directly for their rates of repeated appearance is an extremely time-consuming, tedious process, for the experiments must be performed with the autosomes. MORGAN, BRIDGES and STURTEVANT list cases of allelomorphism which have been found. The frequency of these cases is undoubtedly less than it should be when compared with the visibles which have been rediscovered, due to the technical difficulties just cited. The results so far are only useful in showing that recessive lethals do occasionally reappear.

The evidence seems to justify the assumption that the re-mutating value of the lethal factors is of the same order of magnitude as that for the visible genes. This assumption is utilized in the further discussion of the results which flow from this analysis of the experimental data.

DISCUSSION

The various lines of evidence may now be collected and examined in the light of the problem in hand,—the number, size and characteristics of the genes in Drosophila. Accepting the foregoing analysis as an essentially correct, if rough, description of the changes which take place in chromatin when Drosophila sperm are exposed to X-ray, and accepting the fact that there are approximately 175 visible, viable sex-linked genes, lead to the following numbers for the rest of the categories. The recessive sex-linked lethal genes are 7.3 times as numerous as the visible genes since in the experiment the ratio was 44 visible genes to 320 recessive sex-linked lethals; or there would be $175 \times 7.3 = 1280$ loci which could be occupied by recessive sex-linked lethals. The dominant visible mutations in the sex chromosome and autosomes appeared with a frequency comparable to that of recessive visible sex-linked factors, 175. The rate at which the sex-linked recessive mutations were produced was 0.0_4123 for each electrostatic unit. The rate at which the dominant lethal mutations in the sex chromosome were observed was 0.0_592 for each electrostatic unit. On a random chance distribution of these loci within this chromosome, the numbers of these two categories should be as their rates or 1.0:0.75. The probable number of loci which could be occupied by dominant lethals in the sex chromosome is $1280 \times 0.75 = 960$.

The relation of the numbers of loci in the sex chromosome to the numbers in the autosomes may be determined from the equations given earlier for the decline in the numbers which survive and from the decline in the sex ratio.

These data are gathered together in table 3. *Alpha* represents the number of quanta hitting the chromatin at a given time; *alpha* prime is the number of these quanta which are effective. As the data point to the conclusion that one quantum hit is sufficient to produce a lethal mutation, it follows that α'/α represents that proportion of the chromatin which is composed of these factors as related to the whole. The copper and chromium series are averaged for the volumes of chromatin occupied by the dominant lethal loci.

TABLE 3

	WHOLE SPERM CHROMATIN		SEX CHROMOSOME	
	COPPER	CHROMIUM	COPPER	CHROMIUM
Calculated α	1.163	1.405	0.199	0.240
Observed α'	0.024	0.020	0.0011	0.0013
Relative volume α'/α of vital gene chromatin	Average 0.0175		Average 0.00557	

Obviously the percentage of chromatin in the whole sperm capable of producing dominant lethal mutations is considerably greater than that found in the sex chromosome. This would seem to mean that the gene containing chromatin in the sex chromosome is less than that of the autosomes since the other possible hypothesis, that the average gene size in the sex chromosome differs from that in the autosomes, seems less likely. The ratio of these two values 0.00557/0.0175 would consequently be the amount of gene-containing material found in the sex chromosome as compared with the autosomes, or 32.4 percent.[7] This figure finds important confirmation in the work of STERN, PAINTER and DOBZHANSKY. By cytological study of the size of translocations involving chromosome fragments of known genetic length, PAINTER showed that the gene-bearing chromatin of the sex chromosome was only about 1/3 the length of the whole. STERN concludes that this same length is 1/2 the chromosome. DOBZHANSKY, on the other hand, making a similar study of second and third chromosomes, reached the conclusion that while the genes are unevenly scattered as in the sex chromosome they are found along the chromosome's entire length. These data consequently furnish a direct proof of these conclusions. The effective chromatin area of the sex chromosome is 9.8 percent of that of the autosomes as derived from the relation 5.27×10^{-9} cm$^2\times0.325\div17.51\times10^{-9}$ cm$^2=9.8$ percent. From this relation the number of autosomal loci carrying genes capable of mutating into dominant lethals would be 960/0.098 or 9800 loci. The loci which would contain genes capable of mutating to recessive lethals would be even greater than this, 13100 loci, since the number of

[7] If we calculate this percentage on the chromatin rather than the whole sperm this value is 26.5. Both percentages are subject to fairly large probable errors.

dominant loci is 0.75 that of the recessive loci. The number of loci which are capable of mutating to visible recessive factors of fair viability is, on the basis of sex chromosome proportions, 1800. These estimates of loci number are listed below to facilitate comparison.

TABLE 4

TYPE OF LOCI	NUMBER	PERCENT
Sex linked loci of visible factors	175	0.6
Sex linked loci of recessive lethal factors	1280	4.7
Sex linked loci of dominant lethal factors	960	3.5
Autosomal loci of visible recessive factors	1800	6.6
Autosomal loci of recessive lethal factors	13100	48.0
Autosomal loci of dominant lethal factors	9800	36.0
All chromosome loci of dominant visible factors	175	0.6

The numbers of these loci are considerably greater than we have been accustomed to visualize. The number 1975 for the visible recessive factors is similar to MULLER'S estimate except that in his estimate he considers this number as the whole number of factors,—due partly, no doubt, to the lack of information on lethals at that time. This list of factors does not include factor changes whose only expression may have been in the internal organs. This class of loci is likely to be rather small, however, since most physiological or other changes which have been noted have been accompanied by alterations in structure, which are visible externally on close examination.

The total number of loci within the Drosophila sperm should not be thought of as the sum of the different categories in table 4, for it seems likely that genes belonging to more than one category can occupy the same locus. We know this to be true in connection with dominant and recessive genes. It seems equally probable with regard to lethal genes since in allelomorphic series the viability of these genes is by no means the same, some being practically lethal while others are of nearly wild-type viability. The number of loci which could be regarded as the minimum is consequently 1280 for the sex chromosome and 13,100 for the autosomes. The proportion of the various categories gives the proportion of the different types of genes which may be expected to appear under the action of an agent capable of making the genes mutate.

This tabulation demonstrates that the animal's own genetic constitution is very significant from the standpoint of pathology. It suggests that there are not less than 14,380 loci occupied by genes which are vital to the normal morphology and well-being of the organism. A single factor may cause the destruction of the legs of the fly through development of joint lesions containing melanotic pigment, or it may make the fly pin-headed in ap-

pearance and inviable, etc. Yet these visible signs are but as the red flag of the auctioneer outside the shop announcing greater things going on within. Our study of the mechanism by which the animal's own genes prevent development of pathological conditions lags far behind the studies of pathogens and the lesions they make. Yet if all the external agencies which are known to produce such changes be tabulated they fall far below the possibilities of the organism's own genes in this direction.

The estimate of the number of genes and of the volume of chromatin occupied by these genes as furnished by the X-ray results make it possible to estimate the size of an average gene. Three independent estimates of the volume of the chromatin occupied by the different classes of genes are available. The first relates to the volume occupied by the loci containing genes capable of mutation to dominant lethals; the second to like loci of the sex chromosome; and the third to loci of the sex chromosome containing genes capable of mutating to recessive lethals. The numbers of genes of each class are all estimated from the same value, 175, for the sex-linked loci of the rather viable, visible, recessive genes. These estimates for the different volumes are shown below.

	SENSITIVE VOLUME	VOLUME OF CHROMATIN OCCUPIED	VOLUME OF LOCI
Dominant lethals (sperm)	0.0175	1.17×10^{-14}	1.1×10^{-18} cm^3
Dominant lethals (sex chromosome)	0.00557	1.00×10^{-15}	1.0×10^{-18} cm^3
Recessive lethals (sex chromosome)	0.00752	1.35×10^{-15}	1.0×10^{-18} cm^3

The sensitive volume is calculated from the methods and measurements given earlier, that is, by utilizing certain physical hypotheses. It is well to remember these hypotheses in interpreting the conclusions drawn. X-rays are assumed to be absorbed as units. An absorbed unit has a sphere of effect within which it acts. It is this purely physical quantity which is being measured. The result is biologically significant in that it sets the upper limit of the size of those elements whose alteration produces the observed results. The upper limit for the size of the gene is 1×10^{-18} cm^3. This value is below microscopic vision and therefore considerably less than the chromomeres assumed by BELLING to represent genes.

The possible configuration of the space relations may be obtained from the fact that the genes within the chromosome seem to follow one another as beads on the leptotene thread. The length of the sex chromosome which carries such genes is 0.50×10^{-4} cm. If we consider that every gene is capable of mutating to a recessive lethal, the total number of genes in this thread is 1280. At the leptotene stage this thread is much longer than it is in the condensed chromosome. The condensation could conceivably take place by either of two processes. The thread could contract to the length of the condensed chromosome or the thread could fold on itself in this manner

but keep its original length. If condensation by contraction took place the lineal dimension of a gene locus would be 0.50×10^{-4} cm $\div 1280$ or 3.9×19^{-8} cm. The measurement of the sensitive volume of 1×10^{-18} cm^3 then leads to the depth and breadth dimension of 5.0×10^{-6} cm.

The data on organic crystals show the interatomic distance to vary between 1A and 3A. The dimension of the gene locus 3.9×10^{-8} cm would allow but two atoms in the lineal dimension, a value which appears too low especially when the depth and breadth dimensions are so much larger. It seems preferable therefore to regard the condensed chromosome as composed of a folded thread of leptotene dimensions where the gene locus is rectangular or spherical. Such an assumption leads to a gene 1×10^{-6} cm on a side or there would be 50 atoms on a side of such a figure with 125,000 in all. The average protein molecule has a molecular weight of about 120,000. The average atomic weight of such a molecule approximates 15, or there will be about 8000 atoms in such a molecule. The measurements of the gene consequently make it possible that the gene is composed of something like 15 protein molecules. The results consequently make it probable that the gene size may be reduced beyond this limit. The use of ultra-violet light for the production of mutations may offer a means to this end since the critical volume in which it is absorbed is less than that of X-rays. The difficulty which lies here, however, is that of accurately measuring the ultra-violet absorption by the sperm.

SUMMARY

The significant points brought out in this paper may be summarized as follows. X-rays impinging on cell chromatin cause changes at multiple foci. These changes are heritable. They fall into 3 somewhat overlapping categories, dominant lethals, recessive lethals and viable mutations producing visible somatic effects. The killing effect of X-rays on the first cell generation comes from the production of dominant lethal mutations. The death of cells of the second generation comes from the recessive sex-linked lethals produced. The rates at which the changes are produced follow specific mathematical laws so closely that they may be used to measure the X-ray dosage which is given. The rate at which these changes are produced is dependent on the ionization value in air of the delivered irradiation. The characteristic wave length of the given X-rays seems to play no part in the effects produced.

From the standpoint of those interested in pathology, the most significant findings are those showing the genetic organization of this presumably rather simple animal. The number of separate inheritable loci within such an animal is in the neighborhood of 14,380. The proportions of the genes which occupied these loci were such that

48 percent were autosomal dominant vital factors,
36 percent were autosomal recessive vital factors,
6.6 percent were autosomal recessive factors producing visible effects,
4.7 percent were sex-linked dominant vital factors,
3.5 percent were sex-linked recessive vital factors,
0.6 percent were sex-linked recessive visible factors,
0.6 percent were sex-linked and autosomal dominant visible factors.

The importance of this comparison is the fact that within any animal there are a large number of genes which have the capacity for modification and when so modified produce definite visible pathologies or death. The protective and developmental mechanism which the animal has set up through its evolution is, thus, composed of many parts. This does not seem so strange when it is realized that each and every one of these 14,380 gene loci go to every cell in the body and that were all other tissues of the body removed the body could be seen as a shadow with its present form and structure in the genes which are left.

The proof that the chromatin is not all loci-containing is a significant fact as many workers believe that the presence of thymonucleic acid is diagnostic for such material. In this light, chromatin elimination as noted in tissue has often been misinterpreted as the direct throwing off of a portion of the inheritance. The results presented show that such need not be the case. They offer an explanation for the reported observations that in bacteria and yeast chromatin like that of the higher forms is absent.

The measurement of the size of the gene seems to be the first step in the study of the organization of these genes. The data are such that we can arrive only at a maximum size. This size is 1×10^{-18} cm.[3]

Consideration of the consequences which follow from regarding the condensed chromosome as a condensed leptotene thread or as a folded leptotene thread seems to favor the fold view. Adopting this hypothesis, the gene could be composed of as many as 15 protein molecules of average atomic size.

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