THE INFLUENCE OF CHRONIC IRRADIATION WITH GAMMA-RAYS AT LOW DOSAGES ON THE MUTATION RATE IN *DROSOPHILA MELANOGASTER*[1]

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THE influence of radiation of short wave length on the mutation rate in Drosophila has been measured repeatedly since the pioneer work of MULLER (1927). As a general rule it was found that the mutation rate is directly proportional to the dose of radiation, as expressed in r units. This linear proportionality between radiation dose and mutation rate applies to all dosages of X-rays tested to the present time except for the highest dosages, in which a "saturation effect" comes into play. At the low end of the curve, SPENCER and STERN (1948) found the proportionality maintained down to a dose of 25 r.

It was furthermore found that at high and medium dosages the mutation rate was independent of the intensity, that is, of the time over which the application of a certain number of r units was spread. This was established by PATTERSON (1931) and OLIVER (1932) and others for X-rays, and by HANSON and HEYS (1929, 1932) and RAYCHAUDHURI (1939) for gamma-rays. TIMOFÉEFF-RESSOVSKY and ZIMMER (1935) have calculated that in all experiments a dose of about 3600 r would result in a mutation rate of ten sex-linked recessive lethals per 100 treated sperms.

The experiments reported in this paper have been undertaken in order to examine the question of whether or not the rule that the mutation rate is independent of the time of irradiation also holds for low dosages. While it is well established that at high and medium dosages the same number of r units induces the same number of mutations whether it is applied at once ("acute irradiation") or spread over a considerable time ("chronic irradiation"), this question has not been investigated at very low dosages.

MATERIALS AND METHODS

The methods were chosen in such a way as to be comparable to those used by SPENCER and STERN in their experiments on acute irradiation at low dosages. There were, however, a certain number of differences.

The irradiation was done with gamma-rays, as contrasted with SPENCER and STERN'S X-rays. A needle containing ten mg of radium was used as the

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source of irradiation. The flies to be irradiated were kept in shell vials of glass 7 cm long and 16 mm interior diameter which were closed at the top by gauze attached by a rubber band. These shell vials were arranged on a wooden rack in a semicircle around the radium needle at a distance of 27.5 cm. According to calculation, the dose received in this way would be 2.5 r units per day. This value was checked by DR. WILLIAM F. BALE with a Victoreen dosimeter, and a value of 3.3 r units per day was found. A later check done by the senior author with a different Victoreen dosimeter gave 2.3 r units per day. Within the error of the measurements it seems therefore, that the theoretical value of 2.5 r units per day is correct.

The flies, *Drosophila melanogaster*, were kept during the experiment in an incubator of Celotex which was cooled by a compressor unit and heated by two 60 Watt bulbs controlled by a thermostat. The temperature was kept constant in this way at $18° \pm 1°$C.

The controls were kept in an incubator of the same size and build at $18° \pm 1°$C. The control incubator was cooled by the same compressor as the experimental incubator, so that any disturbance in the compressor unit would affect both experimentals and controls in a similar way. The only possibility of a difference between experimentals and controls was a failure of the bulbs used as heating units. For this reason, both incubators were checked twice every day to determine whether or not the bulbs were still working.

Since the experimental and control incubators were in the same room, the control incubator was protected from the gamma-rays of the experimental incubator. This was done by means of a lead plate 5 cm thick which was attached at a distance of 4 cm in the back of the radium needle. The distance between the two incubators was 1.4 meters. Radiation in the control incubator was checked by DR. BALE with a Victoreen dosimeter and found to be 0.03 r units per 24 hours, that is, one percent of the radiation the experimentals received. The control flies were kept in glass shell vials identical with those for the experimentals, and placed in the incubator on a semicircular rack identical with that used for the experimental animals, except that no radium needle was present.

The breeding prodecure was similar to that used by SPENCER and STERN.

The effect studied was identical with theirs, that is, lethal mutations in the X chromosome of the strain Canton Special. For purposes of testing this strain was bred to the strain Muller-5 which also was used by SPENCER and STERN and which was kindly supplied by DR. H. J. MULLER. This strain has an X chromosome marked by the dominant gene Bar (*B*) and the recessive gene apricot (w^a). Crossing over with the homologous Canton Special chromosome is inhibited by the inversions, sc^8 and *In S* contained in the Muller-5 X chromosome, one of which includes the other.

Virgin Muller-5 females not more than 16 hours old were isolated every morning. Fifty females were mated in half pint bottles to 100 Canton Special males. During most of the course of the experiment, the males had been mated before for at least five days to their Canton Special sisters. This was done because DR. H. J. MULLER had advised us of his results indicating that

the spontaneous mutation rate of the first sperm discharged by a young male is higher than in the sperm formed later in life. While the first experiments were done on males which had not been aged, there is no error introduced by this fact into the experiment, since it is identical for both experimentals and controls. Actually no significant difference between the control mutation rates in the first experiments in which the age of the males was not controlled and in the later ones using aged males was found.

The Muller-5 females were permitted to remain with Canton Special males for four days. After they had been inseminated, carrying Canton Special sperm in their spermathecae, they were etherized and prepared for irradiation. The preparation for irradiation consisted in placing the females into the shell vials mentioned above which contained food in the form of a specially prepared honey-yeast agar slant and a piece of tissue paper to absorb excess moisture. The vials, each containing 50 female Muller-5 flies with Canton Special sperm in their spermathecas, were then transferred into the incubators. One half of the vials were placed into the control incubator, one half into the experimental incubator. In putting the vials into the experimental incubator, care was taken that the agar slant was directed toward the outside away from the radium, so that the gamma-rays did not have to penetrate the agar before reaching the flies.

The flies were exposed to the radium in the incubator for 21 days, so that the final dose of radiation received was 52.5 r. The dose received by the controls in the same interval was about 0.6 r units. During these 21 days the food was changed once, both in the experimentals and the controls, by shaking the flies without etherization into new shell vials containing newly made-up medium, on the tenth or eleventh day.

The choice of honey-yeast agar was made after preliminary tests with several media. The medium required was one that would inhibit the flies from laying eggs, but still allow them to survive. The survival was increased by keeping them at the low temperature of 18°C; the egg laying was inhibited to a certain degree by relatively dry conditions induced by the addition of tissue paper and by the use of gauze in closing the shell vials. This precaution was necessary in order to inhibit the flies from expending the sperm stored in their spermathecae.

Four different media were tried: molasses agar, honey agar, yeasted honey agar, and yeasted cornmeal molasses agar. The results obtained with these four media are given in table 1. Survival of the flies was not included, but it should be stated that with every medium 50–80 percent of the flies were lost, partly by death, and partly by escape while changing the medium.

After having been aged in one of the four media for 21 days at 18°C without irradiation, the surviving females were etherized and placed singly into one ounce creamers containing yeasted cornmeal molasses agar. These creamers were kept at 25°C for two weeks, and the failures, as well as the flies hatching in the single fertile creamers, were counted.

As far as the ability of females to produce any offspring is concerned, honey yeast agar was superior to the three other media used. The highest fecundity as

judged by number of offspring was found after aging on cornmeal molasses agar. Since cornmeal molasses agar offered better conditions for the development of molds during the aging process, and since only a limited amount of flies from each culture was desired for every breeding experiment, yeasted honey agar, containing ten g carrager, 0.5 pint honey, dry yeast and Moldex in one quart of water, was used as the aging medium.

A few remarks are pertinent concerning the causes of the high sterility on all media and the reduced fecundity as compared with non-aged flies. The ques-

TABLE 1

Sterility and fertility of females aged for 21 days at 18°C on different media.

	HONEY AGAR	MOLASSES AGAR	HONEY YEAST AGAR	CORNMEAL MOLASSES AGAR
Sterile females in percent	37.7±1.9	59.0±4.9	31.0±1.7	42.2±2.7
Average number of F_1 females per culture	8.97±0.71	12.12±1.44	12.10±0.42	19.37±1.27
Average number of F_1 males per culture	4.57±0.45	5.08±0.69	6.06±0.24	7.07±0.92

tion of fertility of Drosophila females at different ages has been investigated by HADORN and ZELLER (1943). They found a notable decrease in fecundity in older females; this decrease they proved to be due to the physiological state of the female, not to exhaustion of sperm. It is in agreement with their findings that in our experiments the laying of eggs in the aging vials was never high so that the stored sperm was certainly not exhausted. Females of 21 days of age, according to HADORN and ZELLER, are far advanced on the road to infertility. This is expressed by a decrease in the number of eggs laid of about 40 percent at 20 days and by an egg hatching rate of less than 20 percent, as compared with 60–80 percent in the first two weeks.

After having been aged and irradiated for 21 days, the surviving females were tested for sex-linked recessive lethals.

The aged and irradiated females were placed separately into one ounce or three quarter ounce creamers containing the usual Drosophila food medium, yeasted cornmeal molasses agar with Moldex added, and were kept at a temperature of 25°C.

As indicated in table 1, a large percentage of the cultures were sterile. The number of offspring obtained in different cultures varied. The breeding of isolated aged and irradiated females permitted the study of the influence of radiation on sterility and fecundity. It also had some advantages in diminishing the error introduced by the phenomenon of lethal clusters. This question will be discussed in a later chapter.

When the F_1 animals had hatched, pair matings were made between F_1 females and their brothers or, since there were always less males than females, with Muller-5 males from stock cultures. Not more than 14 females from any F_1 culture were used for mating, a precaution reducing the error introduced by "lethal clusters," as will be discussed later.

The pair matings were made up in three quarter ounce or one ounce creamers with yeasted cornmeal medium. The creamers were kept on trays. Since both the size of the trays and of the creamers varied, the number of cultures per tray varied from 35 to 165. The cultures were kept in a temperature constant cabinet at $25 \pm 1°C$. The cabinet as suggested by WARREN P. SPENCER consisted of five sections of shelves 212 cm high, 93 cm wide, and 30 cm deep. One of these sections was placed against a wall, while the four others formed the sidewalls of the cabinet. They were roofed over with Celotex and insulated towards the outside by a layer of Celotex. The front wall was again made of Celotex and contained a wooden door. The space inside the cabinet was about 137×150 cm, the shelves opening into this space. The cabinet was kept at 25°C by means of a heating element inserted into a motor-driven blower which was regulated by a thermostat.

Keeping the cultures at a constant temperature had two advantages. It kept the cultures at a steady speed of development, so that they were ready to be examined 14 days after they had been made up. Furthermore, the expression of lethals is dependent on external conditions: the same gene may act as a complete lethal under one set of conditions; while under another set some animals carrying the lethal will succeed in surviving. Control of temperature will therefore tend to standardize environmental conditions and make the results more comparable.

After 14 days the hatching cultures were examined for the presence of wild type males. If by simple inspection with a binocular microscope through the walls of the culture jar wild type males were seen, the culture was counted as not lethal. If no wild type males were found in this way, the animals in the culture were etherized, and the animals belonging to the different classes counted. This count was repeated twice at two to three day intervals. If no wild type males appeared during this time, two females heterozygous for the lethal-carrying wild type chromosome were mated in pair matings to their Muller-5 brothers. These pair mating cultures were kept for two weeks in the constant temperature cabinet; then the different classes of animals hatching in the progeny during the third week were counted. The X chromosome was considered as carrying a lethal if no wild type males appeared in F_2, even though in some cases a small number of wild type males appeared in the following generation. If, on the other hand, a single wild type male appeared in F_2, the culture was counted not as a lethal but as a semilethal, regardless of whether or not both cultures in the next generation did contain any wild type males. The only exception to this rule was made when the F_2 culture contained less than 50 animals. In this case, it is possible that no wild types males will be found, purely by chance. Therefore, in this case, the decision as to

whether a culture should be considered as lethal or not lethal was made on the basis of the appearance of wild type males in the next generation.

These criteria are admittedly arbitrary. But since there exist all intergrades between full viability, lowered viability, semilethality, and complete lethality, and since the expression of these characters is also dependent on environmental conditions, distinct criteria had to be set up which were applied to controls and experimentals alike. In the actual experiments, however, most lethals proved to be clear-cut, and only rarely was it necessary to make a decision according to the above-mentioned criteria.

Once a lethal was established, it was kept by mating several females carrying the lethal to their Muller-5 brothers. The lethals were kept in half-pint milk bottles with yeasted cornmeal molasses agar, and were later used for linkage tests.

RESULTS

The results obtained are indicated in tables 2–4. Table 2 contains the sterility in percent, and the average number of males and females produced

TABLE 2

Sterility and fertility of females aged for 21 days at 18°C (controls) and irradiated for 21 days at 18°C with gamma-rays amounting to 52.5 r.

	CONTROLS	NO. OF CULTURES N	EXPERIMENTALS	NO. OF CULTURES N
Percent sterile	41.1±0.25	3988	40.7±0.24	4002
Mean number of females per culture	19.7±0.9	187	19.3±0.8	261
Mean number of males per culture	10.7±0.5	187	11.6±0.5	261

by females aged for 21 days, and by females irradiated during this period with gamma-rays amounting to 52.5 r units. The data do not indicate any significant difference between the experimentals and the controls. There is no evidence, therefore, that the fertility of the females has been affected by the irradiation.

The lethal mutation rates in the experimentals and in the controls are given in table 3. There are added to the table the results of SPENCER and STERN obtained from non-aged sperm, and from sperm irradiated with X-rays of 50 r units. The last column gives the percentage values of which the observed mutation rates would be the upper and lower five percent fiducial limits (five percent in both tails jointly.) These were calculated according to a formula proposed by DR. D. R. CHARLES, as reported by SPENCER and STERN and according to the procedure of STEVENS (1942).

It is seen by comparing lines 1 and 3 that the control mutation rate in

SPENCER and STERN's material was lower than in the experiments reported here. This is due to the effect of aging. This phenomenon had been described previously by RAJEWSKY and TIMOFÉEFF-RESSOVSKY (1939) and by KAUFMANN (1947). By inhibiting males from mating for 20 days, RAJEWSKY and TIMOFÉEFF-RESSOVSKY were able to increase the mutation rate from 0.104 percent in sperm used for fertilization shortly after hatching to 0.263 percent in sperm aged 20 days. Similarly, KAUFMANN found an increase of sex-linked lethal mutations from 0.141 percent to 0.317 percent by aging sperm for 16–17 days. These values are in good agreement with those found in the present

TABLE 3

Lethal mutation rates in the X chromosome of Canton Special sperm, after different types of treatment. (Lines 3 and 4 from SPENCER *and* STERN *1948)*

TREATMENT	NO. OF CULTURES TESTED	NO. OF LETHALS FOUND	PERCENT LETHALS	CALCULATED LIMITS OF PERCENT LETHALS (see text)
Controls aged	56,252	154	.2738	.2356–.3198
52.5 r chronic exposure	51,963	162	.3118	.2692–.3628
Controls not aged (SPENCER and	73,901	72	.0974	.0788–.1220
50 r acute exposure STERN)	31,560	77	.2440	.1985–.3034

experiment. In the present case the sperm were kept in the female spermatheca while aging; the increase of mutation rate seems therefore to be due to aging rather than to place of storage.

Comparison of the mutation rate of sperm chronically irradiated with 52.5 r with that of the aged controls shows that it is somewhat increased in the experimentals. This increase is however, slight, and far inside the 5 percent level of significance. There is therefore in these data no indication that the difference observed between experimentals and controls is not due to chance alone.

This result seems to be in direct contradiction to SPENCER and STERN's finding that acute irradiation with 50 r and even 25 r X-rays causes a significant increase in mutation rate. Since the controls of the two experiments have very different mutation rates due to the aging effect, the experimental mutation rates cannot be directly compared, but only the difference between the mutation rates in experimentals and controls forms a legitimate basis for the comparison of SPENCER and STERN's experiments with those reported here.

The difference observed by SPENCER and STERN between the mutation rates in sperm receiving an acute dose of X-rays of 50 r and their controls is 0.1466 percent. The corresponding difference in the experiments described in the present paper is 0.0380 percent. It is necessary to calculate what the probability is that such a difference might occur by chance.

If it is assumed that the two differences are not significantly different from each other, they must be considered as chance deviations from a universe of differences. As the best estimate for this universe, from which the two values

are chance deviations, DR. D. R. CHARLES has suggested the expression:

$$\frac{\frac{D_1}{SE_{D_1}^2} + \frac{D_2}{SE_{D_2}^2}}{\frac{1}{SE_{D_1}^2} + \frac{1}{SE_{D_2}^2}}$$

in which D_1 and D_2 represent the differences between experimentals and controls found in the present work and in SPENCER and STERN'S experiments respectively, and SE_{D_1} and SE_{D_2} their respective standard errors. By introducing the actual values observed, a value for the best estimate of the difference of 0.0975 percent is obtained.

Taking this value as the most probable difference, expectations can be calculated for the number of lethals and non-lethals to be found in the experimentals and controls in the two experiments. In this way eight values for expectations are obtained for the lethals and non-lethals in experimentals and controls in both experiments. These are compared with the values actually obtained by a χ^2 test (table 3A).

TABLE 3A

Test for homogeneity of the differences between experimentals and controls found by SPENCER AND STERN *and by the present authors.*

	EXPERIMENTALS		CONTROLS	
	m	N−m	m	N−m
SPENCER and STERN				
expected	66.15	31,493.85	82.85	73,818.15
observed	77	31,483	72	73,829
CASPARI and STERN				
expected	178.1	51,784.9	137.9	56,014.1
observed	162	51,801	154	55,998

In this way a χ^2 of 6.1523 for 1 degree of freedom is obtained. This corresponds to a probability of about 0.012. This probability is at a level which is usually regarded as significant.

In table 4, the mutation rates obtained in experimentals and controls during the single months of the experiment are given. Since experimentals and controls were reared and treated side by side, the results obtained in every month can be compared. The monthly mutation rates appear highly variable, both in the experimentals and in the controls. There is, however, no indication that this variability is not due to chance, since no heterogeneity is present in either experimentals or controls. ($\chi^2 = 12.8$ for 8 degrees of freedom, $P = .11$ for the controls; $\chi^2 = 11.5$ for 7 degrees of freedom, $P = .12$ for the experimentals.)

THE IMPORTANCE OF LETHAL CLUSTERS

The phrase "lethal clusters" will be used to designate the occurrence of a number of lethals in the progeny from the same mating. It is interpreted as

indicating the occurrence of a lethal mutation early in the course of spermatogenesis. If a lethal mutation occurs in an early spermatogonium, all X-bearing sperms derived from this spermatogonium will contain the lethals and transmit it to the next generation. The earlier in spermatogenesis the mutation occurs, the larger will be the number of cultures in F_2 which will form a lethal cluster.

Lethal clusters have been disregarded in earlier work on mutations, since they are negligible compared to the high mutation rate induced by radiation

TABLE 4

Lethal mutation rate in the X chromosome of Canton Special sperm, obtained in different months.

MONTH	EXPERIMENTALS			CONTROLS			DIFFERENCE EXP.-CONT.
	NO. OF TESTS	NO. OF LETHALS	PERCENT LETHAL	NO. OF TESTS	NO. OF LETHALS	PERCENT LETHAL	
October				7,022	16	0.2279	
November	6,715	22	0.3276	5,320	11	0.2068	+0.1208
December	6,441	14	0.2174	6,008	14	0.2330	−0.0156
January	5,223	10	0.1915	5,466	9	0.1647	+0.0268
February	7,694	31*	0.4029	6,626	17	0.2566	+0.1463
March	6,540	26	0.3976	5,974	15	0.2511	+0.1465
April	7,165	15	0.2094	6,414	18	0.2806	−0.0712
May	6,770	23	0.3397	6,772	29	0.4282	−0.0885
June	5,415	21	0.3878	6,650	25	0.3759	+0.0119

* Lethal cluster of 9 lethals included.

at high dosages. But in working with low mutation rates, this effect cannot be disregarded, since, if absolute numbers of mutations of about 150 are compared, two or three lethal clusters of three or four lethals, or one larger lethal cluster, may disturb the results considerably. For this reason, SPENCER and STERN considered this phenomenon in their experiments at low dosages, and discussed its occurrence.

In the present investigation precautions have been taken to avoid the danger of lethal clusters: the irradiated P females were isolated, and not more than 14 of the F_1 daughters of each female were tested for lethals. Actually, the number was frequently even lower, since in many F_1 cultures less than ten females were produced. This procedure limits the size of any lethal cluster to 14 at the most.

Furthermore, the individual F_1 daughters of the same irradiated female were always placed in neighboring creamers on the same tray. This arrangement would immediately call attention to the occurrence of a lethal cluster. Since in the experiments at a mutation rate of 0.25−0.3 percent only one lethal on the average was found on three to four trays examined, a large number of lethals on the same tray and in the same neighborhood would always arouse suspicion.

Finally, unless some extremely large lethal clusters occurred, the rate of lethal clusters in experimentals and controls should be similar, since mature sperm were irradiated in the spermatheca of the female. This is a difference between the experiments described in this report and those of SPENCER and STERN. These workers irradiated males which carried both sperm and spermatogonia. A lethal mutation induced by radiation in a spermatogonium would give rise to a cluster of lethals. Since in the experiments being reported no spermatogonia were irradiated, no lethal clusters could have been produced by radiation. All lethal clusters, both in controls and in experimentals, must have been produced before the aging and irradiation of the sperm.

TABLE 5

Expectations for trays containing different numbers of lethals occurring by chance, compared to the actual observed ones.

NO. OF LETHALS	NUMBER OF TRAYS			
	CONTROLS		EXPERIMENTALS	
	EXPECTED	OBSERVED	EXPECTED	OBSERVED
1	107.68	87	124.48	101
2	12.56	18	16.40	17
3	0.978	3	1.44	3
4	0.057	1	0.095	1
5	—	0	0.005	1
6	—	0	—	0
7	—	0	—	0
8	—	0	—	0
9	—	0	7.95×10^{-9}	1

The actual frequency of occurrence of lethal clusters can be estimated. Designating as a "lethal association" all cases in which two or more lethals were found on the same tray, it may be asked: how many associations of two, three, four, etc., lethals will be expected to occur by chance, taking the lethal mutation rates from table 3, and the averages of 84.5 fertile cultures per tray for the experimentals, and 85.2 cultures per tray for the controls. In this way expectations for the occurrence of two, three, etc. lethals on the same tray can be derived from the formula for the Poisson series.

In table 5, the expectations calculated in this way are compared with the distribution of lethals on the trays actually observed. While in the experimentals the whole material has been used, the control data are based on 49,700 observations (that is, 88.35 percent of the total), among which there were 136 lethals (mutation rate 0.2738 percent). This is due to the fact that at the beginning of the experiments in which only controls were tested, the distribution of lethals on the trays was not recorded.

From table 5 it appears that in the experimentals there occurred one lethal association of nine. Its occurrence by chance is highly improbable. Actually,

these lethals occurred in two neighboring rows on the same tray, that is, the nine lethals were found in one sample of 27 cultures. It seems very likely that they constitute one mutational step which occurred early in spermatogonial development. The two lethal associations of five and four lethals each may also be lethal clusters. There is no reason to assume anything but chance association for the occurrence of two and three lethal associations on one tray, though one or two of these groups may also constitute lethal clusters. Linkage tests, to be described below, did indeed suggest that some of the associations of two and three lethals represented clusters.

In the controls, there are no unexpectedly high associations on the trays, except for one tray with four lethals which may possibly constitute a lethal cluster. Otherwise, there may be some lethal clusters of three or two lethals involved. This is suggested by the results of the linkage tests. This difference between experimentals and controls must be due entirely to chance, since the mutational steps in question must have occurred before irradiation.

It is not legitimate to correct for the lethal clusters derived in this way. But it may be suggested that, if the groups of four, five and nine lethals are regarded as one mutational step each, a decrease in the experimental mutation rate from 162 in 51,963 to 147 in 51,963 (=.2829 percent) would be obtained. If the controls were corrected in a similar manner, assuming for good measure that the association of four lethals, two of the associations of three lethals, and six of the associations of two lethals are lethal clusters, a corrected lethal mutation rate of 123 in 49,700 tests (=.2475 percent) would be obtained. The difference, 0.0354 percent, would be of the same order of magnitude as in the uncorrected data.

Illegitimate though this attempt at correcting for lethal clusters may appear, it serves well the purpose of demonstrating that these corrections will not increase the difference between experimentals and controls, since the larger lethal clusters were found in the experimentals.

Another method by which the clustering of lethals may be estimated is the determination of their position on the chromosome. If two lethals are differently situated on the chromosome, they must be due to independent mutation events. If they are at the same place, they may or may not be identical mutations.

For this reason, a number of mutations which had arisen on the same tray were tested by linkage experiments. Female heterozygotes for the lethal were crossed out to males from a stock whose X chromosome was marked by the genes *sc*, *cv*, *v*, *f*, and *car*. F_1 females were mated singly in creamers to their brothers. In F_2, the different recombinations appearing among the males were counted. On the whole, 35 control lethals and 36 experimentals have been examined by linkage tests.

The linkage experiments did not yield any unexpected results. As in the linkage experiments of SPENCER and STERN, the average map length in these experiments was somewhat larger than in the BRIDGES map, mainly because the *cv-v* segment was increased. An unexpected increase in certain map regions

was observed in some of the lethals, both in experimentals and in controls. This same phenomenon has been described by SPENCER and STERN. No interpretation can be given. In any case, it cannot be a result of the irradiation, since it also occurred in the controls.

One control lethal, *lc94*, showed a considerable shortening of the map. It measured 40.2 units, as compared with 67.5 units in SPENCER and STERN'S standard map. This would seem to suggest the possible occurrence of a spontaneous inversion. Unfortunately, the stock was lost, so that no cytological tests could be carried out.

In the experimentals, the large lethal cluster of nine was investigated by linkage tests. Eight of the nine lethals were tested and showed that the lethal was situated between *cv* and *v*, 11.4 to 15.7 units to the right of crossveinless. One of the lethals of this cluster was lost.

In the experimental lethal association of five lethals, all were situated between *v* and *f*. Four of them were only slightly to the left of *f*, between 1.9 and 6.5 units, while the fifth was situated much farther to the left of *f*, 17.0 units. The four lethals near *f* may well constitute a lethal cluster, while it seems doubtful that the fifth one belongs to it. Of the lethal association of four lethals, three were all situated in the short region between *f* and *cv*, while the fourth was lost. Of one of the three associations of three lethals each, all three lethals were situated in the same region of the chromosome, while of the other two associations of three lethals only two were investigated, while the third was lost. In both cases the two lethals were in the same region of the chromosome. Of the seventeen associations of two lethals, twelve were investigated. Seven of these were far removed from each other, so that they cannot possibly be derived from the same mutated cell. The remaining five associations contain two lethals each in the same region of the chromosome.

Of the control lethals, the association of four lethals was tested. One of the four was lost, while the remaining three were in the same region of the chromosome, 9.7, 9.0 and 5.4 units to the right of *v*, respectively. The control lethal associations of three lethals each had two of the lethals in the same region of the chromosome, while the third one was in all cases definitely removed from it. Finally, twelve of the eighteen lethal associations of two lethals each were investigated. Six pairs of them were certainly not identical mutations, while the other six pairs were near enough to allow the possibility that they may have been lethal clusters.

These observations bear out the conclusions drawn from statistical considerations that the experimental association of nine and four lethals constitutes a lethal cluster, that is, that each one constitutes only one mutational step. The same is true for at least four lethals from the association of five, while the fifth remains doubtful. With the associations of two lethal-carrying creamers on the same tray, the same conclusion is not justified. While occurrence in different regions of the chromosome excludes the possibility that the two lethals may be due to the same mutational step, occurrence in the same region does not prove it. This is well indicated by the two control lethals *lc29* and *lc30*. Although they are both situated slightly to the right of *v*, the death in *lc30* occurs

in the pupal period, while in *lc29* it occurs earlier. This difference in developmental effect excludes the possibility that both lethals may be due to the same mutation.

A reliable estimate of the actual influence of lethal clustering is not possible with either of the methods used. But it must be concluded that correction for lethal clustering will not tend to increase the difference between experimentals and controls. Suppose that in the controls (table 4) all associations of lethals are counted as clusters, but that in the experimentals only the clusters of 9 and 4 (confirmed by linkage tests) are corrected for. The difference between experimentals and controls still would be only 0.0443 percent, that is, much lower than the difference found by SPENCER and STERN at 50 r. Since the assumption that all lethal associations in the controls were due to clusters is highly unlikely, it must be concluded that the difference found in the original experiments probably approximates the real difference.

DISCUSSION

The results show with a high degree of probability that the mutation rate obtained in the investigation reported in this paper is lower than that obtained by SPENCER and STERN on the same strain with the same dose of radiation. In order to explain this difference, all differences in experimental technique between SPENCER and STERN's and our investigations will be discussed separately. They are, in short: (a) temperature, (b) aging, (c) wave length, (d) intensity of radiation, (e) plastic versus glass container.

a) In the experiments reported in this paper, during the irradiation the flies were kept at 18°C, while SPENCER nad STERN's irradiations were done at room temperature, that is, probably at around 24°C. Since temperature is known to affect many biological processes, this factor cannot be neglected. The influence of temperature on radiation-induced mutations has been studied repeatedly. TIMOFÉEFF-RESSOVSKY (1934) and MULLER (1940), working with temperatures of 10°C against 35°C, and 5°C against 37°C respectively, did not find any difference in mutation rate induced by X-rays. KING (1947) obtained an increase in mutation rate at 0°C as compared to room temperature.

It seems therefore unlikely that the lower mutation rate obtained in the present experiment is due to the lowering of the temperature to 18°C. Besides, in order to explain the difference obtained in the two mutation rates found, a Q_{10} of about 4 must be assumed. In photochemical processes the Q_{10} usually found lies between 1.01 and 1.07. If therefore the temperature difference should be responsible for the effect observed, secondary processes with a high Q_{10} would have to be postulated. It should be stated that TIMOFÉEFF-RESSOVSKY (1940) has found a similarly high Q_{10} (5.7 for *D. melanogaster*, 3.00 for *D. funebris*) for "spontaneous" mutations.

b) SPENCER and STERN irradiated males, and their sperm were used for fertilization immediately after irradiation. In the experiments reported here, the sperm were stored in the spermathecae of the females, and therefore subject during irradiation to an aging process. That this aging process may actually influence mutation rate by itself, is indicated by the excess of control mutation

in our experiments as compared to those of SPENCER and STERN.

To explain the difference in frequency of induced mutations between SPENCER and STERN's and our results by the difference in the age of sperm would imply that the physiological state of the sperm cell influences the mutation rate under the influence of radiation. There are actually data in the literature which seem to support this assumption.

KNAPP (1939) and KNAPP and KAPLAN (1942) could increase the mutation rate induced by X-rays in eggs and pollen of *Antirrhinum major* by immersion into water. This they explain by the assumption that the amount of hydration of the cell colloids influenced the mutation rate induced by X-rays. This is borne out by the fact that the effect can be reversed by drying the seeds after hydration. HOLLAENDER and SWANSON (1947) found an increase in the X-ray-induced mutation rate of Aspergillus by pretreatment with infrared rays. KAUFMANN and GAY (1946), on the other hand, did not obtain an increase of X-ray-induced sex-linked mutations in Drosophila after pretreatment with near infrared rays. These investigations leave no doubt that the physiological state of the irradiated cell may influence the rate of transmittable changes produced by radiation. There is therefore no reason to exclude *a priori* the possibility that the aging of the sperm cells in the spermathecae reduces the mutation rate produced by irradiation.

OFFERMANN (1939) claimed to have found an increase in X-ray-induced mutation rate in aged as compared to non-aged Drosophila sperm, while TIMOFÉEFF-RESSOVSKY (1931) did not find any influence of aging on X-ray-induced mutation rate in Drosophila sperm. OFFERMANN's experiments seem to indicate that the effect of aging on induced mutation rate would result in an increase of mutation rate rather than in a decrease.

c) The experiments reported in this paper were carried out with gamma rays while SPENCER and STERN had been working with X-rays. The number of ionizations produced in this way was equal, as expressed by the number of r units. A difference between these two types of radiation could be imagined to arise in two different ways. Gamma-ray quanta have a higher energy than X-ray quanta and therefore a lower number of quanta is absorbed to cause the same effect as expressed in number of ionizations. Furthermore, the pattern of ionizations will be different, gamma-rays causing a more scattered track of ionizations than X-rays.

Since the ionizations due to X-rays tend to occur close to each other, the number of cells subject to ionizations will be smaller than in the case of gamma-rays. Assuming that one ionization in a sensitive volume is sufficient to cause a mutation, the mutation rate produced will be dependent on the number of ionizations per cell. The number of ionization events per cell will therefore be compared for SPENCER and STERN's experiment and for the present experiment.

In both cases, about 50 r units were given. Since 1 r unit corresponds to the production of 1.62×10^{12} ion pairs per g tissue, 50 r would produce 81×10^{12} ion pairs per g tissue. According to GOWEN and GAY (1933) the sperm head of Drosophila is 7.36×10^{-4} cm long and 0.368×10^{-4} cm wide. Its weight would

therefore be in the neighborhood of 1×10^{-12} g. This would lead to the conclusion that in both SPENCER and STERN'S and the present experiments 81 ion pairs were produced on the average in each sperm head.

The specific ionization of gamma-rays and medium soft X-rays is different. X-rays of a wave length of 4.1Å units form 2.7×10^6 ion pairs per cm of track, while gamma-rays form 1.5×10^5 ion pairs per cm of track (LEA, HAINES and BRETSCHER 1941). This would mean 270 ion pairs and 15 ion pairs per μ respectively. The consequence would be that, if the mutation rate induced were dependent on hitting a certain sensitive volume, the total number of ionizations being the same, the chances would be better with the use of gamma-rays than with the use of X-rays. In X-rays, due to the closer spacing of the ion pairs, a number of them would be wasted by falling into an identical sensitive volume. This consideration would lead us to the conclusion that gamma-rays should be more rather than less efficient than X-rays; this is contrary to the results.

The possibility remains that the difference between the results obtained by SPENCER and STERN and those in the present investigation is due to differential absorption of quanta. Since one quantum of gamma-rays contains more energy than one quantum of X-rays, a smaller number is absorbed to cause the same amount of ionization, as expressed in r units. If a mutation were dependent on the absorption of one quantum, X-rays should be more effective than gamma-rays. But this conclusion is contradicted by all experiments done at higher dosages in which no differences were found in mutation rates produced by the same dosage of X-rays of different wave length and gamma-rays (MULLER 1940, 1941).

A third possibility must be taken into consideration: the higher absorption of X-rays as compared with gamma-rays by elements of high atomic weight. GRAY (1940) studied this effect for sulfur, and he explained the greater effect of X-rays over gamma-rays of the same dose in r units found by MOTTRAM and GRAY (1940) in the mouse for skin lesions induced by radiation by the higher absorption of X-rays in the keratin which is rich in sulfur. In radiation effects on genes, a similar effect must be seriously considered, since chromosomes are rich in phosphorus. According to MIRSKY and POLLISTER (1942), sperm of different species contains three to six percent phosphorus. Because of the high absorption of radiation by the heavy phosphorus atoms, the actual radiation received by irradiated sperm heads should be considerably higher than that usually estimated for soft tissue. However, no experimental evidence for the higher absorption effect of phosphorus atoms on X-rays has been found in experiments with high dosages.

d) In SPENCER and STERN'S experiment the flies were exposed to the whole dose in a short time, while in the experiments described here the same dose was spread over 21 days. The intensity of radiation per minute was about 1/6000 to 1/13,200 of that used by SPENCER and STERN, depending on whether a dose of 10 r or 22 r per minute was given by SPENCER and STERN.

Influence of intensity on the effect of a radiation has been reported for numerous effects such as killing of bacteria and killing of animal eggs. In all

these cases sigmoid killing curves were obtained. This is in contrast to the effects previously obtained in experiments on mutations, where a direct proportionality between dose and mutation rate was always found, independently of the time factor.

This difference between "physiological" radiation effects and mutation effect has been explained by the assumption of presence or absence of recovery.

It is assumed that in physiological effects the injury done by the primary ionizations can be compensated for, if the original injury is sufficiently small, and if sufficient time is at the disposal of the cell. It is conceivable that the same is true for mutations; and that the dosage which makes recovery possible has to be as low as 1/600 r per minute. This possibility and its implications will be discussed later.

e) Finally, it must be considered that SPENCER and STERN kept their flies during the irradiation in plastic containers, while in the experiments reported in this paper glass shell vials were used. It is probable that the glass vials, because of their silica content, would absorb more radiation than the plastic vials.

In order to obtain an estimate of how much of the radiation was absorbed by the glass walls, ionization chambers of the shape and size of a fountain pen were exposed to the gamma-rays used in the experiments inside and outside a test tube of a wall thickness similar to that of the shell vials. Five different ionization chambers were used, and they were exposed for either 30 or 50 minutes. Fifteen measurements without the glass test tube were made, and seven measurements inside the glass tube. The results obtained with the different dosimeters were in close agreement with each other.

With the ionization chambers exposed directly to the gamma-rays, a mean value of 0.097 ± 0.002 r per hour was found, while from the weight of the radium and the distance a dose of 0.104 r per hour would be expected. The ionization chambers contained in the glass tubes showed a mean value of 0.084 ± 0.002 r per hour, 0.013 r per hour less than the unprotected ionization chambers. This would point to an absorption of 13.4 percent of the radiation by the glass wall. The actual dosage received by the flies was therefore about 45 r rather than 50 r. This correction is not used in this paper, since similar corrections have not been obtained for SPENCER and STERN'S data nor for the other data described in the literature. SPENCER and STERN'S plastic capsules were probably less absorbent, but the soft X-rays may have been more readily absorbed than the highly penetrating gamma-rays. At all events, the absorption by the glass is certainly not sufficient to account for the difference between SPENCER and STERN'S experiment and the one reported here.

While the analysis up to this point excludes the possibility that differential absorption by the container may have been responsible for the different results obtained by SPENCER and STERN and by ourselves, the other possibilities suggested are still more or less likely.

A further analysis is possible by comparing the data reported here to those of RAYCHAUDHURI (1939, 1944). This author irradiated inseminated Drosophila females with low doses of gamma-rays for 30 days at 8°C. He studied

the production of sex-linked lethal mutations by a breeding procedure similar to that used in the present experiment. The results obtained by him and by MULLER (1940) are given in table 6.

TABLE 6

Lethal mutations induced in the X chromosome of Drosophila by X-rays and gamma-rays at different intensities (after MULLER *1940 and* RAYCHAUDHURI *1939, 1944).*

TYPE OF RADIATION	DOSAGE PER MIN. IN r	TIME OF EXPOSURE	TOTAL DOSAGE CA.	NO. OF CULTURES	NO. OF LETHALS	PERCENT LETHALS ± S.E.
X-rays	250.00	8 min.	2000	6224	373	5.99 ±0.27
γ-rays	0.01	720 hrs.	400	3855	59	1.53 ±0.20
γ-rays	0.05	720 hrs.	2000	868	58	6.675±0.85
γ-rays	0.85	45 hrs.	1300	762	40	5.25 ±0.82
None				3471	11	0.317±0.095

The data given in table 6 show a picture quite different from that obtained in the present study. In both cases 2000 r produced mutation rates around six percent, whether treatment was given as X- or gamma-rays and in 30 days or 8 minutes. Furthermore, 400 r produce roughly one fifth of the mutation rate at 2000 r. The results of MULLER and of RAYCHAUDHURI are therefore in complete agreement with the rule that the mutation rate is directly proportional to the dosage as measured in r units and independent of the wave length employed and of the intensity.

Since RAYCHAUDHURI's experiments were executed with a technique very similar to that used in the present studies, certain factors which have been considered as being different in SPENCER and STERN's and the present experiment seem unlikely to be the cause for the difference in the results. Since both RAYCHAUDHURI's and the present experiments were done with gamma-rays, the difference of gamma-rays versus X-rays in itself cannot be the reason for the different results obtained by SPENCER and STERN and in the present report. Furthermore, since RAYCHAUDHURI worked at 8°C, a temperature effect seems unlikely, unless an unusually high stability of the genes at 18°C, declining both towards higher and lower temperatures, were assumed.

The aging effect, however, does not seem to be excluded, even though RAYCHAUDHURI aged his sperm for 30 days, instead of 20 days in the present experiment. For it is quite possible that the physiological aging process is disproportionately retarded at 8°C as compared with 18°C.

Finally, the dosages and intensities given must be compared. The lowest total dose given by RAYCHAUDHURI was eight times as high as that used in the present experiment (400 r versus 50.5 r). The dose given per minute was six times as high (0.1 r/min. against 0.017 r/min.) and the time of exposure was 50 percent higher. The question is whether this difference can account for the difference observed in the experiments.

As mentioned previously in the experiments reported in this paper, every sperm head received on the average 81 ion pairs in the course of the experi-

ment, that is, in 21 days. If the ionizations were distributed equally over the whole period, this would amount to one ion pair every 371 minutes, or about every six hours.

In RAYCHAUDHURI'S experiments, a total of 400 r units was given corresponding to an average of 648 ion pairs per sperm head. One ion pair per sperm head would therefore be found every 67 minutes, or about once every hour.

Actually these rate values are too high, since the ionizations do not occur singly but in groups. Gamma-rays form 1.5×10^5 ion pairs per cm of track (LEA, HAINES and BRETSCHER), or about 15 ion pairs per μ of track. Since the diameter of the head of Drosophila sperm is about 0.3μ, and its length about seven μ, the single ionization events will consist on the average of perhaps five to ten ion pairs per sperm head. This would mean that in RAYCHAUDHURI'S experiments actually one ionization event (group of five to ten ionizations, in the average) occurred every five hours per sperm head, or 72–144 single events per sperm head during the course of his experiment (MULLER 1941). The corresponding values for the experiment reported here are that one group of five to ten ionizations occurred per sperm head every 30 hours, and that the average number of ion pair groups formed during the experiment per sperm head was between about eight and 17.

In all these estimates a number of factors have been neglected, especially the higher degree of ionization due to the phosphorus contents of the chromatin. But these calculations serve to show that the factor of 8 by which RAYCHAUDHURI'S and the present experiments differ involves a change in the order of magnitude of such a kind that the single events in the single sperm head, being only 17 on the average, would not be predictable with a high degree of certainty from statistical laws.

In spite of that, this difference cannot explain the small difference between experimentals and controls found in the present series under the classical hit theory as elaborated by TIMOFÉEFF-RESSOVSKY, ZIMMER and DELBRÜCK (1935), even under its broader formulation by FANO (1942). This is due to the fact that it is a basic assumption of the theory that "the experimental law (1) is valid beyond the present stage of investigation, i.e. is valid down to any low dosage and there is no dependence whatsoever on the time distribution of irradiation" (FANO 1942, p. 245).

FANO'S analysis leaves open the possibility that interaction of "energy transfers" may occur. He mentions two kinds of interactions, "concentration effects", that is, cooperative actions of two or more different adjacent ionizations or energy transfers, and quantum mechanical interference phenomena. In either case the independence of simple ionization events postulated by the classical hit theory would be abandoned. The reduction in difference between experimentals and controls found in the present investigation would necessitate the assumption either that a threshold for the action of radiation exists, or that two or more independent primary events are required for a mutation to occur. The dependence of a mutation on several primary events could again be imagined under different assumptions, such as interaction at the molecular

level, or at a cellular level as assumed in the recovery theory of "physiological" radiation effects.

CONCLUSIONS

The experiments reported in this paper have shown that the difference between the mutation rates of irradiated and non-irradiated sperm is, with a high degree of probability, smaller if the irradiation is distributed over 21 days than if the same dose is given at once. A number of factors which may be responsible for this result have been discussed.

While it seems unlikely that the wave length of radiation, the temperature and the chemical constitution of the containers were the decisive factors, the aging of the sperm has not been excluded as a possible cause for this result, particularly if it is assumed that the mutation-inducing activities of radiation and of aging are not simply additive. It must also be considered that a large number of small effects, such as the difference between glass and plastic containers, may have added up to obscure an actually significant difference between experimentals and controls. Finally, it must be kept in mind that there are still 1.2 chances in a hundred that SPENCER and STERN's and our results have deviated from each other by chance only. This probability, though low, is not by any means an impossibility. On the other hand, if the relation between X-ray dosage and mutation rate is a linear one in general then SPENCER and STERN's result fits the expectation very closely. The probability of our data being a chance deviation from such an expected value is lower than the one given above.

Before accepting the dependence on the time factor of radiation effects on mutation rate at low doses, it will be necessary to exclude all the factors discussed above which may have depressed the mutation rate in our experiments. If the result turns out to be correct, it would necessitate a revision of the classical hit theory of induction of mutations. On the other hand, it would bring the production of mutations into line with a number of other effects of radiation, such as the killing of bacteria and of Drosophila eggs.

SUMMARY

1. The rate of lethal sex-linked mutations in Drosophila exposed to gamma-rays of 2.5 r units per day through 21 days (total 52.5 r) was determined.

2. In a total material of 108,215 chromosomes tested, no significant difference between experimentals and controls was found.

3. The difference between experimentals and controls found in the present investigation is significantly different ($P = 0.012$) from that found by SPENCER and STERN with the same dose of X-rays given at once.

4. By comparison of the present results with those obtained by SPENCER and STERN and by RAYCHAUDHURI it is concluded that it is unlikely that the relative inactivity of the irradiation is due to wave length, temperature or glass containers.

5. As possible causes for the inactivity of irradiation in the present experi-

ment, different sensitivity of sperm at different stages of aging, and dependence of induced mutation rate at low dosages on a time factor are considered.

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LITERATURE CITED

FANO, U., 1942 On the interpretation of radiation experiments in genetics. Quart. Rev. Biol. **17**: 244–252.

GOWEN, J. W., and E. H. GAY, 1933 Gene number, kind, and size in Drosophila. Genetics **18**: 1–31.

GRAY, L. H., 1940 Physical investigation of the contribution of the photoelectrons from sulphur to X-ray ionization. Brit. J. Radiol., N.S. **13**: 25–30.

HADORN, E., and H. ZELLER, 1943 Fertilitätsstudien an *Drosophila melanogaster*. I. Untersuchungen zum altersbedingten Fertilitätsabfall. Arch. Ent. Mech. **142**: 276–300.

HANSON, F. B., and F. HEYS, 1929 An analysis of the effects of the different rays of radium in Drosophila. Amer. Nat. **63**: 201–213.

1932 Radium and lethal mutations in Drosophila. Further evidence of the proportionality rule from a study of the effects of equivalent doses differently applied. Amer. Nat. **66**: 335–345.

HOLLAENDER, A., and C. P. SWANSON, 1947 Modification of the X-ray induced mutation rate in fungi by pretreatment with near infrared. Genetics **32**: 90.

KAUFMANN, B. P., 1947 Spontaneous mutation rate in Drosophila. Amer. Nat. **81**: 77–80.

KAUFMANN, B. P., and H. GAY, 1946 Frequency of recessive lethals induced in Drosophila by near infra-red and X-rays. Anat. Rec. **96**: 34–35.

KING, E. D., 1947 The effect of low temperature upon the frequency of X-ray induced mutations. Genetics **32**: 161–164.

KNAPP, E., 1939 Mutabilität und physiologischer Zustand. Naturw. **27**: 839–840.

KNAPP, E., and R. KAPLAN, 1942 Beeinflussung der Mutationsauslösung und anderer Wirkungen der Röntgen-Strahlen bei *Antirrhinum majus* durch Veränderung des Quellungszustandes der zu bestrahlenden Samen. Z. I. A. V. **80**: 501–550.

LEA, D. E., R. B. HAINES, and E. BRETSCHER, 1941 The bactericidal action of X-rays, neutrons and radioactive radiations. J. Hyg. **40**: 1–16.

MIRSKY, A. E., and A. W. POLLISTER, 1942 Nucleoproteins of cell nuclei. Proc. Nat. Acad. Sci. **28**: 344–352.

MOTTRAM, J. C., and L. H. GRAY, 1940 The relative response of the skin of mice to X-radiation and gamma-radiation. Brit. J. Radiol., N.S. **13**: 31–34.

MULLER, H. J., 1927 The problem of genic modification. Verh. V. int. Kongr. Vererbungsw. **1**: 234–260.

1940 An analysis of the process of structural change in chromosomes of Drosophila. J. Gen. **40**: 1–66.

1941 Induced mutations in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **9**: 151–165.

OFFERMANN, C. A., 1939 Effect of aging on the frequency of induced mutations. Genetics **24**: 81–82.

OLIVER, C. P., 1932 An analysis of the effect of varying the duration of X-ray treatment upon the frequency of mutations. Z. I. A. V. **61**: 447–488.

PATTERSON, J. T., 1931 Continuous versus interrupted irradiation and the rate of mutation in Drosophila. Biol. Bull. **61**: 133–139.

RAJEWSKI, B. N., and N. W. TIMOFÉEFF-RESSOVSKY, 1939 Höhen-Strahlung und die Mutationsrate von *Drosophila melanogaster*, Z. I. A. V. **77**: 488–500.

RAYCHAUDHURI, S. P., 1939 The validity of the Bunsen-Roscoe law in the production of mutations by radiation of extremely low intensity. Proc. 7th Int. Cong. Gen. Edinburgh 246.
1944 The validity of the Bunsen-Roscoe law in the production of mutations by radiation of extremely low intensity. Proc. Royal Soc. Edinburgh, **62**: 66–72.

SPENCER, W. P., and C. STERN, 1948 Experiments to test the validity of the linear r dose mutations rate relationship at low dosages. Genetics **33**: 43–74.

STEVENS, W. L., 1942 Accuracy of mutation rates. J. Gen. **43**: 301–308.

TIMOFÉEFF-RESSOVSKY, N. W., 1931 Einige Versuche an *Drosophila melanogaster* über die Art der Wirkung der Röntgen-Strahlen auf den Mutationsprozess. Arch. Entw. Mech. Org. **124**: 654–665.

1934 Einige Versuche an *Drosophila melanogaster* über die Beziehungen zwischen Dosis und Art der Röntgenbestrahlung und der dadurch ausgelösten Mutationsrate. Strahlen-Therapie **49**: 463–478.

1940 Eine biophysikalische Analyse des Mutationsvorganges. Nova Acta Leopoldina, Halle **9**: 209–240.

TIMOFÉEFF-RESSOVKSY, N. W., and K. G. ZIMMER, 1935 Strahlengenetische Zeitfaktorversuche an *Drosophila melanogaster*. Strahlentherapie **53**: 134–138.

TIMOFÉEFF-RESSOVSKY, N. W., K. G. ZIMMER, and M. DELBRÜCK, 1935 Über die Natur der Genmutation und der Genstruktur. Nachr. Ges. Wiss. Göttingen (Math.-Phys. VI,. Biol.) N. F. **1**: 189–245.