$$\frac{v}{V} = \frac{(1 + b - \beta/2)b}{2(b - \beta/2)}\left(\frac{\tau - \bar{\tau}}{E}\right),$$

where V is the volume of the bar itself. Corresponding to the approximations (10) and (11), we now have the relations

$$\frac{v}{V} = \frac{1 + b}{2}\left(\frac{\tau - \bar{\tau}}{E}\right); \quad \frac{v}{V} = \frac{1}{2}\left(\frac{\tau - \bar{\tau}}{E}\right).$$

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*THE RELATION BETWEEN X-RAY DOSAGE AND THE FREQUENCY OF SIMULATED HEALING OF CHROMOSOME BREAKAGES IN DROSOPHILA MELANOGASTER FEMALES**

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Communicated by H. J. Muller, May 12, 1954

X-radiation produces breakages in the proximal chromatin of attached-X chromosomes in germ cells of female *Drosophila*,[1] thus resulting in "detachment" of the X's. In the most commonly found type of case, detachment involves recombination of the heterochromatin of the X with the Y,[2] but Rapoport[3, 4] reported obtaining detached X's from females which had attached X's but contained no Y. Although he did not explain how he had achieved the production of attached-X females without a Y, they were later obtained by the cross of males of Lindsley and Novitski's stock, in which the X has both parts of the Y attached[5] to females with attached X's, and the production of detached X's by the resulting Y-less attached-X females was fully confirmed.[6, 7] The detached X's thus produced, when recovered in the next generation, are stable, containing a centromere, and can persist indefinitely. Presumably the acentric arms are lost. It is likely that the centric arms which are recovered represent only a fraction of those produced, since it is to be expected that sometimes a centric arm, after dividing, would have its daughter chromosomes join at the now-duplicated point of breakage, producing a dicentric isochromosome which eventually resulted in a bridge and thus in the death of the cell.

Two hypotheses have been presented to account for those cases derived from Y-less females in which the centric arm persisted. Either the broken ends healed, i.e., formed new stable telomeres,[3] or the cases simulating new telomere formation were actually the result of segmental interchange.[7] In the interchange process a telomere-bearing subterminal region of another chromosome (an autosome) would usually become joined to the centromere-bearing portion of the broken attached-X chromosome. In the case of the fourth chromosome, however, the portion which became attached to the arm of the X could be long enough to include, on occasion, the centromere of the fourth, but in that case the arm of the X involved would be the acentric one. Likewise, in a minority of cases involving chromosomes 2 or 3, the acentric arm of the X might become substituted for so small a subterminal re-

gion of the autosome that the deficiency of this region, simultaneously produced in heterozygous condition, would not kill the offspring having the "detached" X. The loss of the parts complementary to those surviving would in some cases occur by the breakage-fusion-bridge cycle and in others by their becoming segregated to a different pole from the surviving parts at some meiotic or mitotic division. In other papers[6-8] other evidence to test the adequacy of the interchange hypothesis is given, based on the nature of the products formed.

The present experiments were designed to attack the problem from a different angle. On the former hypothesis, that of adaptive telomere formation, the frequency of healed breakages would be expected to increase in a linear manner with increase in X-ray dosage, since the frequency of breaks themselves is proportional to dose, while according to the latter view, that of interchange, the frequency of rearrangements, each requiring the simultaneous occurrence of two or more breakages for their production, would be expected to increase as an exponent of the dose distinctly higher than 1 as is known to be the case with ordinary translocations.[9, 10] Accordingly, females were treated with two different dosages of X-rays, and the numbers of exceptional F_1 individuals of appropriate types were determined.

Materials and Methods.—Virgin females of the "snoc" attached-X stock, having no Y chromosome, carrying sc ct^c oc car on one of the arms of the X and y In49 sn^{x2} on the other, were used. The normal alleles of sc and y serve as markers for the ends of the X chromosome arms which carry them, except for a tiny terminal region distal to these markers (see Fig. 1). The females were collected within a period of 12 hours after their eclosion. After being aged for 3 days more, the females were divided into nine groups, one of which was used in control crosses, four of which were simultaneously given about 4000 r of X-rays, and the remaining four of which, all equal in number; were given about 1000 r by treating each one during a different quarter of the treatment time for the larger dose. By this means it was assured that the average X-ray dose administered was precisely four times less in the flies treated with the smaller dose than it was in those given the larger one. Immediately after irradiation the females were mated in bottles with y sc^{s1} B In49 v/Y^+ males. The presence of recessive y and sc alleles here allow the dominant y^+ and sc^+ markers of the chromosome ends of the females' attached X's to manifest themselves in offspring bearing the father's X and part of the mother's attached X's. In an attempt to obtain not very different degrees of crowding, and therefore of selection for or against exceptions, in the cultures representing different doses, the numbers of female and male parents introduced per bottle were, respectively, 150 and 300 for 4000-r cultures, 30 and 60 for 1000-r cultures, and 17–30 and 30 for the controls. The parents were transferred to new bottles after two days and were discarded at the end of the fourth day after irradiation. The crosses were carried out at 25 ± 1° C.

Results.—The results of nine series of experiments, each containing treatments with 1000 r and 4000 r as well as controls, are summarized in Table 1. Figure 1 shows in simplified form the X-chromosome composition of the parental and F_1 flies, so far as shown by their phenotypes, but omits all representation of the autosomes and parts of autosomes which may have been involved. In the F_1 of the control crosses there were 5078 unexceptional "snoc" females and 47 females produced by nondisjunction in the male parent, but only 135 males (all unexceptional, having

the paternal X). Even though one-half of the fertilized eggs regularly fail to develop, being composed of equal numbers of eggs with three X chromosomes and

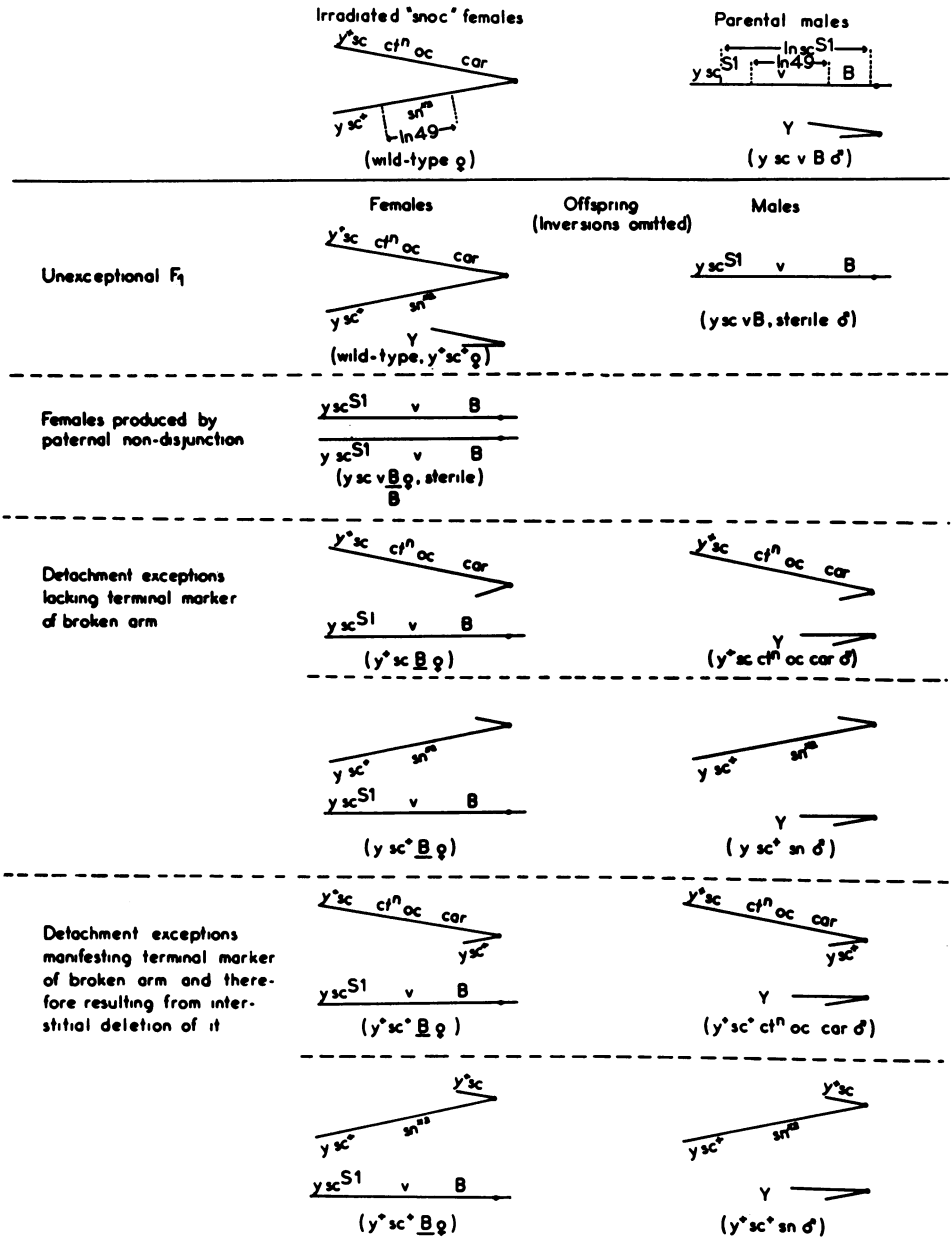

FIG. 1. Simplified diagram of cross, showing only X and Y chromosomes and chromosome parts Besides the mutant genes used as markers, their dominant normal alleles are also indicated, in the case of the loci y and sc. (Phenotypes are given in parentheses below gentoypes.)

eggs with none, the individuals in the one-half which may develop have very unequal chances for survival as adults. For, while the developing unexceptional F_1

females have a wild-type phenotype, the y sc^{S1} B v phenotype of the developing F_1 males is so detrimental that very few survive as adults after competition with their sisters. The mean number of 85 (5078/60) for unexceptional female survivors per bottle in the controls must represent an initial developing population of more than 170 individuals per bottle. No exceptional males or females, indicating spontaneous loss of part or all of an arm of the "snoc" attached-X, were obtained in the controls; nor were any triplo-X (wide-Bar) females found.

The developmental conditions in the bottles containing females treated with 1000 r were similar to those in the control bottles, since 90 unexceptional "snoc" F_1 fe-

TABLE 1

F_1 OFFSPRING FROM CROSSES OF y sc^{S1} B In49 v/Y^+ ♂♂ WITH "SNOC" ♀♀ WHICH WERE EITHER UNTREATED OR TREATED WITH 1000 r OR 4000 r OF X-RAYS

	Control			1000 r			4000 r		
	Brood 1	Brood 2	Total	Brood 1	Brood 2	Total	Brood 1	Brood 2	Total
No. bottles	30	30	60	35	35	70	33	33	66
Males:									
ct^n oc*	0	0	0	0	0	0	1	0	1
sc ct^n oc car†	0	0	0	3	6	9	0	6	6
y sn^{x2}†	0	0	0	2	0	2	2	1	3
Unexceptional (y sc^{S1} *B* In49 v)	62	73	135	102	157	259	29	52	81
Females:									
y sc^{S1} B In49 v (paternal X's)	15	32	47	9	34	43	0	15	15
Heterozygous Bar	0	0	0	15	27	42	23	39	62
Unexceptional ("snoc")	1895	3183	5078	1679	4635	6314	391	1000	1391
Per cent exceptional ♀♀ of all ♀♀ (excluding nondisjunctionals)	0	0	0	0.9	0.6	0.67	5.6	3.8	4.26

* Has the y^+ and sc^+ markers representing both ends of the attached X's.

† The class designated as sc ct^n oc car ♂ included one individual not having car, while that designated as y sn^{x2} included 2 having car. This is because during the breeding of the "snoc" stock car sometimes changes places between the arms of the attached X's by crossing over between the right end of In49 and the locus of car, and these crossovers, being unrecognizable phenotypically, gradually accumulated in the stock.

males were produced per bottle. Eleven male exceptions representing detachment of the arms of the X's were found in the 1000-r bottles, in addition to the 259 unexceptional males obtained. Among the females, in addition to the 6314 unexceptional "snoc" F_1 females, there were 42 exceptions representing detachment and 43 produced by paternal nondisjunction. On the other hand, despite the attempt to get a number of offspring per 4000-r bottle that was similar to that obtained for the control and 1000-r bottles, by means of placing larger numbers of parents in the bottles, the average number of "snoc" F_1 females obtained was only 21 per 4000-r bottle. At this dosage, 10 exceptional males representing detachment were formed along with 81 unexceptional F_1 males, and 62 exceptional "detachment" females and 15 produced by paternal nondisjunction were obtained, besides 1391 "snoc" F_1 females.

The frequencies of exceptional individuals in relation to total females other than those produced by paternal nondisjunction, from the 1000-r and 4000-r treatments,

are, among the males, 0.173 and 0.69 per cent, respectively, a ratio of 1:4, and among the females, 0.67 and 4.26 per cent, respectively, a ratio of 1:6.46. The phenotypes of the exceptional females and their frequencies are given in Table 2.

TABLE 2

TYPES AND FREQUENCIES OF HETEROZYGOUS BAR EXCEPTIONAL FEMALES

PHENOTYPE	1000 r		4000 r		4000 r PER CENT/ 1000 r PER CENT
	No.	Per Cent	No.	Per Cent	
y B	14	0.25	27	1.86	...
sc B	12	0.20	25	1.72	...
(Total)	(26)	(0.41 ± 0.081)	(52)	(3.58 ± 0.485)	8.8 ± 2.1
y^+ sc^+ B*	16	0.25 ± 0.063	10	0.69 ± 0.218	2.8 ± 1.1
TOTAL	42	0.67 ± 0.10	62	4.26 ± 0.53	6.46

* The presence of the markers representing both ends of the attached X's shows that in these cases there was an interstitial deletion of one arm.

Discussion.—Before considering the bearing which the frequencies of exceptional individuals following different X-ray treatments have on the mechanism of detachment, it is necessary to discuss how the variation in culture conditions may have influenced the results. Now the half-pint bottles used, containing about 50 cc. of a yeast-enriched food medium, provide the best culture conditions when some 200–300 flies are developing in them. When very many flies, say more than 500, develop in such a bottle, the amount of food is a limiting factor, causing delay in development, reduction in the size of the adults, and death to some of the developing individuals. When very few, say less than 50, animals develop in such a bottle, yeast, molds, and bacteria overgrow the culture so fast, because relatively few individuals are eating and churning the food, that the food pad becomes a very much poorer place to obtain nutrition and to complete development. In the present experiments, the numbers of offspring per bottle were much smaller in the 4000-r cultures than in the control or in the 1000-r cultures. Since the irradiation produces dominant and partially dominant lethal and detrimental mutations (especially, when we consider the attached-X females, aneuploid chromosome abnormalities), this would cause the frequency of the unexceptional males and paternal nondisjunctional females, relatively to the unexceptional females, to rise as the dose rose. The rise in their frequency in passing from the controls to the 1000-r group is, however, mainly an indication of better culture conditions in the latter (more optimal crowding), because that rise is much greater than the rise in their frequency in passing from the 1000-r group to the 4000-r group, even though the increase in dosage is far greater (3×) in the latter case. Now, since the 1000-r bottles produced (somewhat, though only slightly) more flies per bottle than the controls (but more if the dying larvae caused by irradiation are considered) yet had better culture conditions for development than the controls, because of the greater crowding, the 4000-r bottles must really have had *much* worse conditions than the 1000-r bottles, since they had *much* less crowding, as shown by the very small number of flies hatched per bottle.

Because the control and, more especially, the 1000-r cultures were grown under fairly good conditions, while the 4000-r cultures provided poorer conditions for development, one would expect this difference to be reflected in the numbers and types of flies which survived in the two cases. In favor of a better relative viability of "detachment" females over "snoc" females is the fact that the former each carry two X chromosomes, one of which has not been irradiated, while the latter carry

irradiated attached-X's. Therefore, certain types of induced X-chromosome mutations with dominant detrimental effects (mainly those types of aneuploidy in respect to which the two arms are not complementary) will occur oftener in the exceptional than in the unexceptional females. However, there are reasons for inferring that, despite this, "snoc" females have, on the average, viabilities that are superior to those of detachment females. For, while the former have a "wild-type" phenotype, the latter are all heterozygous Bar, some being also either yellow or scute. More important, regardless of which hypothesis for the origin of detachments obtains, all exceptional individuals are to some extent aneuploid and many are highly aneuploid, while this is much less often the case for the unexceptional ones. Moreover, with increase of dose this selective difference adverse to the exceptions would become greater.

For these reasons the frequency obtained by dividing the observed number of exceptional females by the sum of the observed numbers of "snoc" and exceptional females is very probably below the frequency which occurred during the egg stage, and the frequency would fall shorter, relatively to its true value, the higher the dose. Moreover, when the culture conditions are poorer (quite apart from the effect of increased dose in selectively damaging the flies), the value observed would be a greater underestimate of the true egg-stage value than when food conditions are better. Accordingly, it is probable that for both these reasons the observed percentage of exceptions has been reduced by adverse selection more at 4000 r than it has been at 1000 r. This would make the real difference between the frequencies of exceptional females at the two doses actually greater than observed. It may be added that certain tests of the data which have been made lend support to this interpretation.

One comparison of the incidence of surviving exceptional flies at 1000 r and 4000 r can be made, using the frequencies obtained, by dividing the numbers of exceptional males by the numbers of unexceptional and detachment females. It must first be pointed out that these males, because of their recessive markers, have much poorer viability than the unexceptional females and even than the detachment females. Second, the chance for survival of exceptional males is undoubtedly much better in 1000-r than in 4000-r cultures because of the afore-mentioned much better conditions for development in the former than in the latter group. Third, the radiation would have had a far more detrimental effect on the exceptional males, because of their haplo-X condition in which the X had been irradiated (that is, any genetic changes would, so to speak, lie uncovered), than on any of the other classes of flies, and this influence would reduce their collective viability increasingly at higher doses. Thus, to a far greater extent than in the case of the exceptional females, the difference in culture conditions and that in radiation both work in the same direction to reduce the frequency of exceptional males found, relative to that produced, much more at the higher than at the lower dose. The reckoning from the data gives, at 4000 r and at 1000 r, percentages of 0.69 and 0.173, respectively, a ratio of 4:1. In view of the foregoing discussion, the actual ratio would be much larger than this and therefore much larger than the 4:1 ratio of the doses of radiation.

A more nearly correct estimate of the frequency of exceptional flies in these experiments is obtained when the number of exceptional females is compared with

the number of their unexceptional sisters. At 1000 r, 0.67 ± 0.10 per cent of the females were exceptions, while at 4000 r there were 4.26 ± 0.53 per cent. This ratio of 6.46:1 is higher than is consistent ($P \sim 0.02$, i.e., on a fiduciary level of about 1 in 50) with the 4:1 ratio expected, were all, or almost all, the exceptions consequent to single breakage events. This does not yet mean that all or almost all the exceptions were produced after multiple breakages. For, although we know from previous work[7] that one component of the group of exceptional females results from two-break cases, in which a subterminal part of the X, bearing the telomere, joins at its broken end to another breakage point located in or near the proximal heterochromatin of this chromosome, it would still seem possible that a detectable number of the exceptions might be the result of single breakages in or near the proximal heterochromatin followed by healing. If this were the case, then, upon separating the total group of exceptional females into two groups, one known to contain only intra-X interchanges and the other potentially composed, in part, of cases of single breakage followed by healing, additional analysis could be made.

The data in Table 2 show that the group of y B and sc B exceptions which potentially contains single healed breakages constitutes 3.6 ± 0.5 per cent at 4000 r and 0.41 ± 0.08 per cent of the females at 1000 r, being in the proportion of 8.8:1 for the two treatments. Such a ratio is statistically higher ($P \sim 0.01$) than the 4:1 ratio expected if all of these exceptions were the consequence of single breakages. Surprisingly enough, then, this group of exceptions, from which support for healed breakages would be most apparent, actually gives stronger evidence to the contrary than does consideration of the whole group of exceptions or of the group of exceptions known to be composed of intra-X interchanges. For this latter group increased from 0.25 ± 0.06 per cent at 1000 r to only 0.69 ± 0.22 per cent at 4000 r, a 2.8-fold increase, which is statistically consistent with ($P = 0.70$), although not evidence for, an increase which is linearly proportional to the X-ray dosage. That exceptions of fundamentally this type, i.e., large deletions of the X, increase in frequency as the $^3/_2$ power of the dose when sperm are irradiated was shown by Muller, Vogt, and Koerner.[10] Evidently, then, either the accident of small numbers or some special circumstance (see below) has caused the observed ratio in our experiment to fall below that which actually obtains. At any rate, no justification remains for considering any of the exceptional females as resulting from single healed breakages.

A possible explanation for the apparently nonexponential increase in the frequency of intra-X interchange females with dosage can be based on the supposition that the hyperploidy of these females, with an extra bit of the X subterminal region, tends to be more damaging than that of the y B and sc B exceptions, which have autosomal hyperploidy, thus resulting in a much lower viability, or at least on the supposition that there is a longer region of the X in question that has a sliding scale of viability (varying greatly with conditions). Consistent with this view is the observation[8] that a large proportion of the autosomal hyperploids involve chromosome 4, and, since one arm of this is entirely heterochromatic and the other known not to cause much inviability when present in triple dose, many of the autosomal hyperploids would be relatively immune to being selectively crowded out by poor cultural conditions. It has been noted in the foregoing that the poorer cultural conditions in the cultures for the higher dose than in those for the lower dose act selectively against flies carrying the most genetic detriment.

Two conclusions may be reached from these experiments. The first is that the data are consistent with the view that all the exceptions produced could well be the result of two or more breakages. This means that the earlier view[3] that a detectable percentage of new telomere formation is found after irradiating females, is no longer tenable, and therefore supports the alternative hypothesis[7] that the results could be due entirely to eucentric chromosomal interchanges.

It has been reckoned[7] that if the chromosomes, at the time the interchanges occurred, were in the tetrad stage, the frequency of interchanges which were phenotypically unidentified (those F_1 females in the present study having sc B or y B phenotypes) would be $9^1/_2$ times as great as that of interchanges which were identified as involving only the X (those represented in our present study by exceptions having y^+ sc^+ Bar phenotypes). This calculation assumed that the subterminal regions of all chromosomes are equally breakable and liable to be included in interchanges the products of which can reach maturity, that the frequency of breaks to the left of scute is so small as to be negligible for these purposes, and that the distribution of the chromosomes among egg and polar bodies is not substantially different for eucentrically interchanged chromosomes of the different types in question, involving the tips of different chromosomes. The calculation also ignored the reciprocal type of case, in which a detached acentric arm of an X became attached in place of the subterminal region of an autosome, which thereby became haplodeficient in that region (a condition severely restricting the size of the piece absent in a viable exception). If such cases, whatever their relative number, were added, the identifiable y^+ sc^+ B exceptions would be outnumbered that much more than $9^1/_2$ times. It seems, however, that one (or more) of the original postulates requires modification, for, although the real ratio of unidentified interchanges is probably higher than the 3:1 in the present experiments (Table 2), it, like the numbers 45:6 obtained in earlier experiments,[7] is probably less than $9^1/_2$:1.

The present data offer no evidence, except in those interchanges which result in the y^+ sc^+ B individuals, concerning the origin of the subterminal regions which joined to the break proximal to the centromere of the X. A number of possible explanations of the departure of the ratio of the unidentified to the identified interchanges from the "expected" $9^1/_2$:1 suggest themselves here, however. It may be that subterminal breakage of the X is higher than it is in the autosomes. More likely is the possibility that the former breaks oftener succeed in accomplishing interchanges with breaks in the proximal heterochromatin of the X than the latter breaks do. One ground for this would be provided if in oöcytes chromosomes were in such relative isolation from one another as to considerably favor intra- over interchromosomal gross rearrangements. Another ground (suggested by Muller) would be the presence of Inversion 49 in heterozygous condition in the attached X's, inasmuch as the loop thus caused would result in the distal region of the X being brought about twice as close to the proximal heterochromatin as the tips of the other chromosomes. Finally, elimination of the X's carrying subterminal pieces of autosomes into the polar bodies might be more frequent than that of X's with large deletions. At the same time, it must be recognized that viable cases of detached X's resulting from the substitution of an acentric arm of the X in place of the tip of an autosome must arise even less frequently than the complementary cases just

considered—as is to be expected, because of the limitations on their viability already mentioned.

The second conclusion reached is that evidence has been obtained, for the first time, for a considerable number of gross chromosomal rearrangements taking place after *Drosophila* females are X-rayed. That interchanges are a frequent type of mutation in irradiated females is evident both from the present study, where more than 4 per cent of interchanges were produced at 4000 r, and earlier ones.[3, 7] Schultz[11] has summarized the almost completely negative results of other early studies to detect chromosome rearrangements after X-raying females. Glass[12] reported only six gross chromosomal aberrations in 2189 tests from females treated with about 2000 r of X-rays. In extensive analyses of changes involving specific loci, occurring in females, no spontaneous gross rearrangements were found,[13] nor were any found after treatment of oöcytes and oögonia with 4600 r, although later oöcytes gave a frequency of presumptive deficiencies (small deletions) at these loci similar to the results from treated spermatozoa and significantly higher than the results with oögonia.[14] The conclusion was reached[14] that chromosome breakage evidently occurs as frequently in late oöcytes as in spermatozoa but that union of fragments occurs fairly promptly, i.e., prior to the movements of the meiotic divisions, so that there is less opportunity for gross rearrangements to be formed.

In the present experiments only flies derived from late oöcytes have been studied. Having learned from the present work that the exceptions obtained earlier[7] were probably all interchanges between broken ends, it can now be stated definitely that the frequency of these interchanges, of gross rearrangement type, is higher when mature oöcytes are treated with X-rays than when younger oöcytes or oögonia are. This is consistent with the results obtained earlier for X-ray-induced deficiencies (small deletions) in these cells[14] but presents a contrast with the situation found in that and other work for gross rearrangements.

Probably the paucity of gross interchanges previously reported is due to the techniques of detection used, if such interchanges, when produced in females, are especially likely to involve breaks in subterminal and in heterochromatic regions or if it is especially likely that, in females, when two broken pieces join, the other two will fail to find and fuse with one another. Since it is exactly cases of these kinds which our own technique was adapted to detect, our data do not bear directly on the frequencies of the more orthodox types of gross interchanges.

The experiments in the present and in an earlier paper[7] do not determine whether or not the detached X's which showed the recessive marker (y or sc) of the distal end of one X represented deletions in which one break was to the left of the given locus, or interchanges with autosomes. Experiments designed to investigate this matter will be reported separately.[8]

Summary.—Irradiation of late oöcytes, having the "snoc" attached-X chromosome and containing no Y chromosome, with 1000 r and 4000 r of X-rays has produced a large number of cases in which the X was broken and one of the arms produced persisted in the offspring. The incidences of such individuals among the F_1 females at these doses were 0.67 ± 0.10 and 4.26 ± 0.53 per cent, respectively. Analysis of these data and of their components shows that there is no basis for believing that the broken chromosomes became stable by a process of new telomere formation following single breakage. The frequencies of those exceptions which

might according to their phenotype, have represented single breakages, being in the ratio of 1:8.8, clearly represent gross chromosomal rearrangements in which eucentric union occurred between one breakage point in or near the proximal heterochromatin on one of the arms of the X chromosome and another breakage point located subterminally, sometimes in the X, but probably more often in other, chromosomes. The present data, indicating that all the exceptional flies may be considered to carry gross rearrangements, make it possible to state definitely what was before only suggested[7]—that gross rearrangements occur more frequently after X-raying oöcytes than after X-raying oögonia. The fact that gross rearrangements have been detected only rarely in other studies after treating oöcytes is probably due to the unsuitability of the genetic methods employed to detect changes of the types occurring in this material.

* This work has been supported by a grant from the United States Atomic Energy Commission (Contract AT[11-1]-195). It is a pleasure to thank Mr. Seymour Abrahamson for his excellent assistance in many phases of this work.

† Contribution No. 557.

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