decrease in the number of offspring per treated female may be responsible for the inflection in the non-disjunction curve, namely, that the offspring which would have shown non-disjunctions is killed off by x-rays in a larger proportion than the regular offspring.

Summary.—At low dosages the increase in the percentage of primary non-disjunctions was found to be almost proportional to the x-ray dosage applied. Between 1200 and 2000 r-units the curve shows a sharp break, the increase in the percentage of non-disjunctions becoming smaller. Since at about the same point the fertility of treated flies begins to decline, it is suggested that at higher dosages the offspring which would have shown non-disjunctions is killed off by x-rays in a larger proportion than the regular offspring.

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[2] Authors are indebted to Dr. H. Fricke for the calculation of the wave-length.

RECOVERY FOLLOWING GENETIC DEFICIENCY IN MAIZE[1]

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Deficiency in Maize.—Chromosomal or sectional deficiency normally occurs with low but measurable frequency in early endosperm development in maize. This is shown in seeds heterozygous for linked endosperm characters by the occasional appearance of endosperm chimeras, in which a portion of the endosperm shows the loss of linked dominant characters present in the remainder. Of the 10 maize chromosomes, 7 may be marked by genes for endosperm characters, five chromosomes by one gene each (*R*, *Su*, *Y*, *Pr* and *A*), one by two linked genes (*Y* and *Bh*), and one by three linked genes (*C*, *Sh*, and *Wx*). Endosperm chimeras for each of the 7 chromosomes have been found in untreated material. The normal frequency varies from about 9 chimeras per 1000 seeds for *A* to less than 2 per 1000 seeds for *Su*. Evidence from chimeras of the *C-Sh-Wx* group indicates that the deficient region is often (possibly always) only part of the chromosome.

X-ray treatment shortly after fertilization not only increases greatly the frequency of deficiency in the young endosperm, as has been reported previously,[2] but induces deficiency in the young embryo as well. In cultures heterozygous for a plant character, irradiation at this stage causes

the production of a small proportion of deficient plants showing the recessive character. These are usually very defective in growth and partially sterile. If the treatment is delayed until some days after fertilization, plant chimeras with deficient sectors result.[3]

Irradiation of pollen also induces deficiency. In untreated *c sh wx* ears pollinated by x-rayed *C Sh Wx* pollen, the loss of the dominant genes in the pollen is shown by the production of a few seeds showing the recessive characters. Most of these are colorless, shrunken, and waxy (*c sh wx*), showing the loss of all three dominant genes, but a considerable proportion have lost *C* (and usually *Sh*) without losing *Wx*. Thus, as in the case of the endosperm chimeras, the deficiency is often, or perhaps always, sectional. Some of the losses of the dominant genes may be due to recessive gene mutation, but if so the proportion is very small. Similar loss of the other endosperm genes is shown in appropriate crosses. Although the distinction between deficiency and recessive mutation cannot be established individually for these cases, because of the lack of linked endosperm genes, it is assumed that these also are due largely to deficiency.

In a similar manner the loss in treated pollen of various dominant genes for plant characters is shown in the plants of the next generation. Deficiencies of 5 genes determining plant characters, *A*, *B*, *Lg*, *J* and *G*, have been found following x-ray treatment. The gene *A*, in the presence of certain complementary genes, determines both endosperm color and plant color. In the cross $a \times A$, treatment of mature pollen induces deficiency of *A* independently in endosperm and progeny, as expected from the fact that the two sperms which are to fuse respectively with the polar nuclei and the egg are separate in the mature pollen grain. When the treatment is applied several days earlier (before the division of the generative nucleus), the seeds with colored endosperm produce colored plants and those with colorless endosperm produce colorless plants.

Recovery in Deficient Endosperms.—Reversion following deficiency was first found in deficient endosperms resulting from pollen treatment. Tassels of an *A R C Wx Pr* stock were treated at various stages of development subsequent to microsporogenesis. As the pollen matured (from 1 to 16 days after treatment) pollinations were made on *a R C pr*[4] and *A R c wx* stocks to detect the loss of the dominant genes *A*, *Pr*, *C* and *Wx* in the pollen. Among 3916 seeds produced by pollination on *a R C pr* ears, 27 were colorless, indicating loss of *A*, and 9 were red, indicating loss *Pr*. In addition, 18 seeds were colorless except for one or more small spots of colored tissue, and 4 were red except for similar spots of purple. The portion of the seed showing the dominant character was most frequently a single spot considerably less than 1 sq. mm. in area. Under low magnification it is seen to consist of a group of aleurone cells ranging in number from about 20 to several hundred. Similar but

smaller spots hardly visible to the unaided eye appear on magnification. In many cases there are two or more spots on a single endosperm, and sometimes as many as 8 or 10. The frequency of endosperms with two or more spots is distinctly higher than would be expected from chance coincidence. On the average the total area showing the dominant character is less than $^1/_{100}$ of the area of the endosperm surface.

If the loss of *A* and *Pr* may be ascribed to recessive mutation, the reappearance of the dominant character may be considered the result of reverse mutation in a cell from which the dominant sector is derived. Since linked endosperm characters are not available in the *A* and *Pr* chromosomes, a positive distinction between deficiency and recessive mutation cannot be established for these cases. But results with the *c wx* crosses show that in these at least the loss and recovery are a chromosomal rather than a genic phenomenon. Among 2374 seeds produced by pollination of *A R c wx* ears with pollen of the treated plants, 27 were colorless (18 colorless waxy and 9 colorless non-waxy). In addition, 5 seeds were colorless waxy except for colored spots similar to those described above. In all 5 cases the colored spots were non-waxy, showing that the chromosome or section carrying *C* and *Wx*, which was absent or inactive in the remainder of the endosperm, was present and normally functional in the cells comprising the small dominant sector. Presumably each spot was derived from a cell in which the deficiency was somehow nullified. The return to normal activity of genes previously deficient is termed "recovery."

An endosperm largely deficient but with a small non-deficient sector might conceivably be a chimera of the type described in the first paragraph, resulting from the loss of a chromosome or section in an early division in endosperm development. Normally a loss occurring in the first division would produce a chimera showing the deficiency in only about half of the endosperm, and later losses would produce smaller deficient sectors. But it is possible that a first division loss may sometimes be followed by such asymmetric development that the resulting sectors of the mature endosperm are very different in size. Among endosperm chimeras in untreated material a few cases are found in which the dominant sector includes only about one-fourth, one-eighth or even a smaller fraction of the entire endosperm. Possibly these are the result of asymmetric development following a chromosomal irregularity in the first division. May not the seeds described above as examples of reverted deficiency be accounted for as more extreme cases of the same sort?

This explanation is inadequate for several reasons. (1) The frequent occurrence of the non-deficient tissue in several unconnected sectors is difficult to account for on the hypothesis of asymmetric development of a first division chimera. If the non-deficient sectors result from the re-

covery of the deficient genes after several cell generations, these may be regarded simply as cases in which recovery occurred in several cells of the same endosperm. (2) The frequency of the deficient seeds with small non-deficient sectors is far too high in proportion to the frequency of other endosperm chimeras on the same ears. The number of such seeds was in fact much greater than that of typical first division chimeras (seeds with approximately half of the endosperm showing deficiency), the respective frequencies being 27 and 12 among the progenies from the tassel treatments described. In untreated material, among more than 100,000 seeds examined (which included 35 typical first division chimeras for *A*, *Pr* or *C Wx*), only 6 deficient seeds with small non-deficient sectors have been found. (3) The frequency of typical endosperm chimeras (including all those in which the deficient sector occupies approximately one-half of the endosperm or less), is not materially higher in seeds produced by the use of x-rayed pollen than in untreated seeds. In other words, although pollen treatment results in a great increase in the frequency of deficient endosperms and endosperms deficient except for a small non-deficient sector, it does not materially affect the frequency of deficiencies presumably arising in the early divisions in endosperm development. There is, however, a moderate but distinct increase in the frequency of the anomalous chimeras in which the dominant sector occupies only one-fourth or one-eighth of the endosperm. This suggests that such chimeras may be, at least in part, the result of induced deficiency in the pollen followed by recovery in early endosperm development.

Recovery in Endosperm Chimeras.—Small areas of non-deficient tissue, similar to those resulting from recovery in deficient endosperms, are sometimes found also in the deficient sectors of typical endosperm chimeras. It is possible that a surface appearance suggesting recovery may sometimes result from the normally irregular process of endosperm development. The sectors in typical endosperm chimeras sometimes have very devious boundaries, and occasionally a narrow peninsula of one sector extends far into the other. A similar outgrowth passing through the interior of the endosperm could, in the case of a chimera for aleurone color, produce an island of colored tissue within the borders of a colorless sector. In the case of deficiencies involving *Wx*, it is possible (by the use of an iodine stain) to trace the outlines of the sectors within the interior of the endosperm as well as upon its surface. In several cases small spots of *C Wx* tissue have been found in *c wx* sectors, and in no instance was there any indication of an internal connection with the large non-deficient portion of the endosperm. In order to account for these spots as displaced portions of the unaffected tissue it would be necessary to postulate some kind of nuclear migration, often occurring at a rather late stage in endosperm development. In view of the evidence for recovery in deficient endo-

sperms, it is much more probable that the small areas of non-deficient tissue found in the deficient sectors of chimeras are the result of a similar recovery of deficient genes.

In the pollen treatment experiments summarized above, the 5 instances of recovery for the *C Wx* chromosome were found among 22 seeds in which both *C* and *Wx* were deficient, and none was found among 9 seeds deficient for *C* and not for *Wx*. This leaves open the possibility that recovery may be limited to cases in which the entire chromosome is deficient. But in a later experiment with pollen treatment, recovery was found in two endosperms deficient for *C-Sh* and not for *Wx;* and in the deficient sectors of endosperm chimeras, several similar instances have been found. In general, recovery appears to follow the deficiencies known to be sectional as frequently as the deficiencies which may involve the entire chromosome.

Recovery in the Sporophyte.—The cases thus far cited involve recovery only in the endosperm. Unless the phenomenon occurs also in the sporophyte it can of course have no effect in inheritance. None of the endosperm genes involved can be detected with certainty in the embryo, in the stocks used in these experiments, but the presence of *A* in the sporophyte can be determined by the anthocyan coloration of the plants of the next generation. In the absence of *A*, anthocyan is entirely lacking. In the cultures used in the study of deficiency and recovery of *A* following pollen treatment, the presence of the complementary plant color factor *B* insured the development of distinct red plant color on all exposed parts (except leaf blades) of the non-deficient plants.[5] From the seeds produced by the use of pollen treated at various stages, 1011 plants were grown, of which 9 were deficient for *A*, and one (plant 10–377.1–1) showed clear evidence of recovery. This plant was wholly green except for several narrow but distinct and sharply delimited stripes or lines of red on the lower leaf sheaths. The total area of the red portion was about $^1/_{50}$ of the area of the affected sheaths, and no red color was found above the fifth leaf. Like most deficient plants, plant 10–377.1–1 was very defective in growth, and in spite of optimum growing conditions it reached only about one-fourth the normal height, tasseled and silked late, and matured no pollen. Pollinated by an "a-tester" (*a C R*), it produced 8 wholly colorless seeds.

Recovery in Untreated Material.—Recovery is not limited to deficiencies induced by irradiation. Several thousand of seeds heterozygous for various endosperm characters (10,590 for *A* and *Pr*, 36,919 for *R*, and 92,551 for *C Wx*) were produced without irradiation in 1929, as material for study of the natural occurrence of endosperm chimeras. These were grown in isolated fields more than a mile distant from that in which the x-ray treatments were applied. Some 700 endosperm chimeras were

found, including for each of the chromosome regions several cases in which one or more small spots of non-deficient tissue occurred in a deficient sector. In addition, as perhaps more convincing evidence of deficiency and recovery, there were 11 seeds in which the entire endosperm was deficient except for one or more small spots of non-deficient tissue (1 seed for *A*, 5 for *R*, and 5 for *C Wx*). In proportion to the total frequency of deficient endosperms, the frequency of deficient endosperms showing recovery was as high in this untreated seed as in the seed produced by the use of x-rayed pollen.

Discussion.—Recovery, therefore, is not a consequence of x-ray treatment or of some special type of deficiency induced by x-rays; it is found more frequently in irradiated material merely because deficiency is more frequent in irradiated material. Recovery occurs in both "normal" and "induced" deficiencies, in deficiencies involving each of the four chromosome regions tested, in deficiencies which are certainly sectional as well as in those which may involve the entire chromosome, and in both sporophyte and endosperm tissue. These facts suggest that the germinal change which results in deficiency may commonly be an event somewhat less decisive than the absolute loss of a portion of the gene-chain. If deficiencies in general are reversible, a mechanism may be provided for the occurrence of apparent dominant "return" mutations without any actually "creative" change in the germ plasm. The possibility of an analogous basis for the behavior of the so-called mutable genes and eversporting characters also merits investigation.

The cytological mechanism resulting in deficiency and recovery is unknown. The term deficiency was applied originally by Bridges[6] to the loss or inactivation of a section of the chromosome, and the definition was later extended to include cases in which an entire chromosome is absent, "clearly due to loss and not to inactivation" (Morgan, Bridges and Sturtevant).[7] In the light of our present knowledge of chromosome behavior it seems extremely improbable that a chromosome may reappear in the progeny of a cell from which that chromosome is absent, but not inherently unlikely that an inactive chromosome may sometimes return to its active state. There is, however, no evidence at present for the existence and continued transmission of genetically inactive chromosomes. Moreover, Dr. Randolph's cytological study of maize plants in which deficiency had been induced by x-ray treatment of either the embryo or the parental pollen showed that these deficiencies commonly involve the absence of a chromosome or of a cytologically detectable chromosome fragment.[8]

A hypothetical mechanism, providing for recovery in deficient tissue in which cytological examination shows the absence of a chromosome, may be outlined as follows: Assume that the radiation or other stimulus

does not cause the destruction or loss of the chromosome, but merely deprives it of the power of reproduction. At each succeeding mitosis this chromosome goes to one daughter cell or the other. It is not necessarily inactivated as a functioning cell-constituent; but so long as it does not divide it will be present in only one cell of the organism, and its activity there will produce no visible effect. If in the course of development it recovers the power of reproduction, the progeny of the cell in which the recovery occurs will constitute a non-deficient sector. If recovery is gradual, the chromosome dividing sporadically for a time before regaining normal regularity, several non-deficient sectors may result. If recovery does not occur or is too long delayed, the individual will be typically deficient, and cytological examination of all but a negligible fraction of its cells will indicate that the chromosome has been lost. This hypothesis is subject to various experimental tests, which have not yet been completed.

Summary.—1. Recovery is the return to normal activity of genes previously deficient.

2. In endosperms, or in sectors of endosperm chimeras, in which deficiency has been induced by x-ray treatment, recovery is manifested by the occasional appearance of small spots of non-deficient tissue.

3. Deficient endosperms and endosperm chimeras occur also (though rarely) in untreated material. Recovery occurs as frequently in the deficient tissue of untreated material as in deficient tissue produced by irradiation.

4. Four chromosome regions (marked by the genes *A*, *R*, *C-Wx* and *Pr*) have been used extensively in experiments on deficiency in endosperm tissue. Several instances of recovery have been found for each of the four regions.

5. Recovery occurs in the sporophyte as well as in the endosperm. Deficiency of *A* in x-rayed pollen was followed in one case by recovery in the young sporophyte, producing a plant deficient except for several narrow stripes of non-deficient tissue.

[1] These experiments were supported in part by a grant from the National Research Council, Committee on Effects of Radiations on Living Organisms.

[2] Stadler, L. J., These PROCEEDINGS, **14**, 69–75 (1928).

[3] Stadler, L. J., *J. Hered.*, **31**, 3–19 (1920).

[4] For the complementary action of the genes *A*, *C*, *R*, and *Pr*, see Emerson, R. A., *Cornell Agr. Expt. Sta. Memoir*, **16** (1918).

[5] For the genetics of plant color in maize, see Emerson, R. A., *Ibid.*, **39** (1921).

[6] Bridges, C. B., *Genetics*, **2**, 445–465 (1917).

[7] Morgan, T. H., C. B. Bridges, and A. H. Sturtevant, *Bibliographia Genetica*, **2**, 3–262 (1925).

[8] Randolph, L. F., in a paper presented before Section O, *Amer. Assoc. Adv. Sci.*, Des Moines, 1929 (unpublished).