Reports

Mutation in the Protozoan Paramecium multimicronucleatum as a Result of X-irradiation

Abstract. Mutations have been produced in the protozoan *Paramecium multimicronucleatum* by repeatedly exposing clonal cultures of the animals to large dosages of x-irradiation. The descendants of x-rayed survivors showed the following changes, which persisted in culture: destruction and loss of all micronuclei, decrease in vitality and reproductive rate, increase in x-radiation sensitivity, monstrosity, and reduced body size.

Mutations of diverse kinds have been reported as a result of x-irradiation in a number of representative free-living flagellate, ciliate, and ameboid *Protozoa*. Striking changes in size of the giant multinucleate ameba, *Chaos chaos*, were produced by x-radiation of selected dividing animals during the latter part of the mitotic process (*1*). Clones were created which yielded amebas of a size (volume) that averaged about 60 percent that of the parent clone. These changes persisted for 4 years, the duration of the experiment. In similar manner, another size change was produced from this second clone which resulted in another 60 percent reduction.

Experiments were initiated in 1954 in an attempt to breed x-ray–resistant clones of *Paramecium multimicronucleatum* (*2*). As a result, it was discovered that the radiations increased sensitivity rather than resistance. Furthermore, amicronucleate progeny were produced, as well as other persisting changes.

The x-ray generator used operated simultaneously two water-cooled Coolidge tubes in alternate parallel. One tube was mounted permanently on a platform, and the other tube was supported on a counterbalanced arm which allowed it to be moved vertically and in line directly over the fixed tube. The animals were thus irradiated by a cross fire of x-rays from above and below. The x-ray tubes operated at 182 kv peak and at 25 ma, with an equivalent filtration of 0.2 mm of copper. When the tubes were brought as close together as possible, maximal intensity was 5500 r per minute.

For each irradiation experiment, 200 paramecia were selected from rich clonal cultures in desiccated lettuce medium and placed in each of four nylon syringes of 2-ml capacity and free of air spaces, then x-rayed simultaneously. Details of the method and its advantages have been described previously (*3*). Immediately after all irradiation exposures, survivors of the different doses were expressed from the syringes into test tubes filled with buffered lettuce medium containing the bacterium *Aerobacter aerogenes*, which served as the food source. Upon regaining reproductive ability, progeny of the survivors were harvested, placed in the syringes, and irradiated as before. In this manner, six approximately evenly spaced irradiation exposures were given within a 33-day period at varied dosages which resulted in bringing the cumulative clonal dosage to 1000 kr.

Survivors and their progeny were cultivated in lettuce medium and, after a lapse of 10 months from the last exposure to x-rays, were similarly irradiated seven times within another 33-day period to bring the total clonal exposure for both periods to 1800 kr. The use of the four syringes at one time provided a convenient means of varying the exposure dosage. For example, paramecia in the first, second, third, and fourth syringes were exposed to dosages of 50, 100, 150, and 200 kr, respectively. For the second exposure, given later to progeny of the survivors of the preceding irradiation, the dosage was increased. Thus, specimens in the first syringe next received 100 instead of 50 kr; those in the second, 150 instead of 100 kr; and so on. In later irradiation periods, progeny of irradiated survivors were generally exposed to 100 to 250 kr, although occasionally doses up to 400 kr were employed.

Control and irradiated animals were stained by means of the Feulgen reaction, after fixation in Schaudinn's fluid.

One conspicuous result of this type of irradiation is the complete destruction of all micronuclei yielding entirely amicronucleate clones of animals. Unirradiated control specimens possessed three (rarely four) vesicular micronuclei, each measuring 2.5 μ. The disappearance of the micronuclei was gradual. Clones investigated cytologically after having received 1000 kr revealed 8 percent with two micronuclei, 32 percent with one micronucleus, and 60 percent amicronucleate. After clonal exposure to a total of 1800 kr, all micronuclei were destroyed (Fig. 1).

The size and shape of the organisms were altered as a result of the irradiation—characteristics which persisted even more than 1 year after the final exposure to x-radiation. Measurements of 50 living control specimens averaged 206 μ (range, 190 to 228 μ), while a like number of the heavily irradiated clonal specimens averaged 144 μ (range, 122 to 175 μ). Instead of being "cigar-shaped" and streamlined, like the controls, specimens from the heavily irradiated clones were broader and ellipsoidal.

Additional persisting characteristics of specimens from clones irradiated in this manner even 1 year after the last x-ray exposure are the following: decreased reproductive rate, greater x-radiation sensitivity, reduced swimming vigor, and occasional occurrence of monstrosity. Data based upon daily isolation of specimens in spot plates showed that the reproductive rate of well-fed control animals occasionally reached three divisions per day, and generally not less than two, while clonally irradiated specimens possessed a fission rate that rarely reached two divisions per day (24° ± 1.5°C).

Animals from clonal cultures previously irradiated with the cumulative dose of 1800 kr were more radiosensitive than those irradiated for the first time.

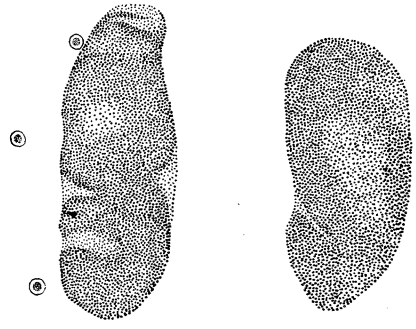

Fig. 1. (Left) Three small vesicular micronuclei to the left of the large, compact macronucleus of a normal unirradiated animal. (Right) Amicronucleate condition showing only macronucleus, since micronuclei have been destroyed as a result of the x-irradiation. (× 600)

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

For example, in one experiment specimens which had been treated in identical manner by irradiation and isolation were then observed at least five times throughout a 48-hour period after exposure to four different doses, ranging from 85 to 343 kr. The 50 control specimens irradiated for the first time with 343 kr showed a survival rate of 58 percent. In contrast, the 31 specimens from the cumulatively irradiated (1800 kr) clone, after exposure to 343 kr, showed a survival rate of 45 percent 48 hours after irradiation.

X-irradiation has been shown to effect micronuclear number in certain other ciliate *Protozoa.* When specimens of *Paramecium aurelia,* which possess two vesicular micronuclei, were irradiated with single doses of from 200 to 537.4 kr, allowed to multiply for 24 hours, and then killed and stained for micronuclear examination, it was reported that the fraction of specimens with less than two micronuclei increased with increase in dosage while the fraction with two—the normal number—decreased (*4*). In the ciliate *Tetrahymena pyriformis,* which has one micronucleus, haploid exconjugants were produced as a result of crossing one clone that was exposed to 400,000 r with nonirradiated cells of opposite mating type. After two haploids were crossed, a clone resulted which showed 80 to 90 percent amicronucleate animals (*5*). When cultures of two different strains of *T. corlissi* were successively and heavily x-irradiated in a manner somewhat similar to that which I first used in 1954 (*2*), amicronucleate races were also produced (*6*).

During the past 30 years, some paramecia collected in nature revealed the amicronucleate condition (*7*). These naturally occurring amicronucleate forms yielded races which have proved to be as vigorous as their micronucleate sisters. It would appear, at least in these instances, that the micronucleus is unnecessary to the life of ciliates which depend upon asexual reproduction alone. Indeed, amicronucleate races can mate and conjugate with micronucleate ones of opposite mating type.

On the other hand, Miyake (*8*) treated dividing specimens of *Paramecium caudatum*—which has but one micronucleus—with urea and created amicronucleate and bimicronucleate races. He reported that loss of all micronuclei was usually followed by considerable decrease in vitality, fission rate, and body size—all characteristics which I found in the irradiated, amicronucleate animals. Miyake concluded that the micronucleus of *P. caudatum* is a fundamental part of the cell and that the increase or decrease of the number of micronuclei causes a physiological imbalance resulting in the accompanying deleterious effects.

In the heavily irradiated clones of *Paramecium multimicronucleatum* discussed here, the attribution of decrease in vigor, size, reproductive rate, and radioresistance to the loss of micronuclei is untenable, since amicronucleate races of ciliate *Protozoa* which have all the normal and vigorous characteristics of micronucleate races occur in nature. The effects induced by x-irradiation in *P. multimicronucleatum* are more probably caused by the alteration of the sets of genes which are located in the macronucleus (*9*). The experiments suggest that subjecting these organisms and their progeny to repeated exposure of x-irradiation yields mutations which are deleterious to the race (*10*).

RALPH WICHTERMAN
Department of Biology,
Temple University, and
Marine Biological Laboratory,
Woods Hole, Massachusetts

References and Notes

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10. This study was part of a project aided by a contract (NR 104–475) between the Office of Naval Research, Department of the Navy, and Temple University and by the Committee on Research, Temple University.

8 September 1958

Ozone in High Concentrations as Cause of Tobacco Leaf Injury

Abstract. Evidence obtained by means of rubber strip tests and an ozone recorder indicates the presence of abnormal concentrations of ozone in the atmosphere at times. Excellent correlation was obtained between appearance of "weather fleck" in tobacco and high values for ozone. The great similarity between lesions occurring naturally and those produced by ozone in chambers also indicates that ozone is the probable inciting agent of weather fleck. Varietal differences exist. Study of stomatal action helped to explain variation in leaf injury.

Tobacco leaf injury known as "weather fleck" or "fleck" has become a serious problem in the production of cigar-wrapper tobacco in the Connecticut Valley (*1*). Fleck has been observed on flue-cured tobacco in North Carolina and in Ontario, Canada, and on other tobacco types in some areas. Since 1954, and perhaps earlier, fleck of varying degrees of severity has developed on tobacco in plots at Beltsville, Md., about 6 miles northeast of the District of Columbia.

Research in 1958 at Beltsville and at Riverside, Calif., provides evidence that the inciting agent of tobacco weather fleck is high concentrations of ozone. Formation of ozone by photochemical reactions involving nitrogen dioxide, certain hydrocarbons, or other air-borne chemicals identified in the Los Angeles area is known to occur (*2*). On tobacco, as on bean (*3*) and grape (*4*), the small lesions occur on the upper leaf surface. Primary lesions are restricted to the palisade layer (*1, 4*). After a day the larger lesions, which usually do not exceed 3 mm in diameter, may appear also on the lower leaf surface. Leaf injury progresses from the bottom to the top of the plant. Normally, leaves are not susceptible until they are fully expanded.

Stretched rubber is used as a specific test for ozone (*5*). To provide tension, the strips of rubber are tied in loop form (*2*). During September, daily exposures of rubber strips were made in the tobacco plots in the hope of correlating fleck appearance with days of high ozone concentration. Ozone concentration based on cracking of rubber strips for each exposure date was evaluated by C. E. Bradley and A. J. Haagen-Smit of California Institute of Technology. From 16 September to 22 October a continuous recorder of atmospheric ozone was operated in the field. This recorder was a prototype of model 725-2 ozone recorder made by Mast Development Corporation (Davenport, Iowa), which was on loan for test purposes to the U.S. Public Health Service (*6*). Although the measurements, based upon the oxidation of potassium iodide solution, are believed to be reliable, further tests are planned to determine the specificity of the instrument for ozone.

Important to these studies were cigar-wrapper varieties of tobacco from the Connecticut Valley. One variety, designated "C" (*7*), is so susceptible to fleck that it cannot be grown for commercial tobacco production. A resistant variety is designated "B" (*8*). Early, medium, and late plantings of the cigar-wrapper varieties were made at Beltsville, both under a cloth shade tent and without a shade tent. During September and October observations were made each day for the appearance of new fleck symptoms, especially in the medium and late plantings. In the early planting, the fleck outbreaks in July and August resulted in lesions on 92 and 64 percent of leaves of varieties C and B, respectively.

Studies conducted in California showed that tobacco was very sensitive to ozone, and the flecks produced by ozone were similar to naturally occurring fleck. The varieties susceptible and resistant to naturally occurring fleck showed 72.0 and 17.5 percent, respectively, of leaves in-