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THE EFFECT OF IRRADIATION ON RECOMBINATION IN ESCHERICHIA COLI

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Gates[1] has reported that the growth processes of *Escherichia coli* are less sensitive to ultra-violet light than cell division. When these organisms are exposed to a limited dose of radiation, they form "spaghetti-like" filaments which attain lengths up to 150 microns and diameters up to 3 times that of normal cells. We have examined these filaments both in the living state with the phase microscope and after staining the nuclear bodies by Robinow's procedure.[2] As the filaments grow it was observed that the nuclear bodies divide regularly and each filament soon contains pairs located 3 to 4 microns apart. After several hours many of the filaments recover from the effect of the ultra-violet treatment and produce normally dividing cells of the organism. In studying the mechanism of this recovery, it was observed that in some filaments a swelling appears near the center and the nuclear bodies migrate to this area and apparently fuse together to form a mass of Feulgen-positive material. The swelling increases in size until it is 6–10 microns in diameter. The nuclear material takes on a granular appearance and a colony of apparently normal organisms will develop at this site on agar plates seeded with irradiated organisms. The appearance of the process is analogous to that observed by Dienes[3] with cultures of a Proteus species. It seems possible that the organism, unable to divide normally, has resorted to a primitive or rudimentary sexual mechanism. We are aware of the pitfalls inherent in cytological studies of microörganisms, and since our photographs were not convincing we have used a cultural procedure for studying the effect of ultra-violet radiation on sexual activity.

A number of *E. coli* mutants were obtained from Dr. Lederberg.[4] These strains are unable to synthesize certain necessary amino acids or vitamins

and, therefore, will not grow in a minimal medium. However, when two strains with different deficiencies are grown together one can isolate from the resultant culture individuals which are prototrophs of the parent strain (i.e., organisms which are identical with the parent strain in their nutritional requirements). Other mutations such as virus resistance or inability to ferment lactose can be tested at the same time. These are distributed in the prototrophs isolated from mixed cultures of mutant strains as recombinations are distributed in higher forms. This suggests that *E. coli* has a life cycle like *Neurospora* with only an occasional sexual phase.

Four-hour broth cultures of the mutant strains were exposed to ultraviolet lamp for 30 seconds and inoculated singly and in combinations into nutrient broth along with similar unirradiated controls. After the cultures were incubated for 6–24 hours, they were washed and plated on minimal medium to detect prototrophs. It will be observed from table 1 that when two strains were grown together which were both B^-M^- (biotin and methionine deficient) in no case did one obtain organisms which could grow on the minimal medium. A similar result occurred when two strains of T^- L^- B_1^- (threonine, leucine and thiamin deficient) organisms were grown together. When two strains with different deficiencies, for example, B^-M^- (which can synthesize threonine, leucine and thiamin) was grown with T^- L^- B^- (which can synthesize biotin and methionine) organisms were obtained which could synthesize all factors (prototrophs of the parent strain) in numbers of about 1 for each 10^7 cells plated as Lederberg[4] has reported. It will be observed from table 1 that the organisms subjected to irradiation produced up to 17 times as many prototrophs as the unirradiated cultures. Yet the irradiation produced no change in the cultures inoculated with single strains or with pairs of strains containing the same deficiencies.

TABLE 1

EFFECT OF IRRADIATION ON RECOMBINATIONS

	IRRADIATED INOCULUM			CONTROL INOCULUM		
ORGANISMS*	TOTAL COUNT	PROTO-TROPHS	RATE/ MILLION	TOTAL COUNT	PROTO-TROPHS	RATE/ MILLION
58-161 + Y10	50M	130	2.6	280	230	0.8
58-161 + Y53	21	160	7.6	220	100	0.45
58-161 + Y87	60	0	0	270	0	0
Y53 + Y10	80	0	0	190	0	0
Y53 + Y87	70	360	5.1	250	100	0.4
Y87 + Y10	90	330	3.7	220	130	0.6
58-161	70	0	0	150	0	0
Y53	50	0	0	340	0	0
Y10	70	0	0	160	0	0
Y87	110	0	0	120	0	0

* 58-161 = B^-M^-; Y10 = $T^-L^-B_1^-$; Y53 = $T^-L^-B_1^-Lac^-$; Y87 = $B^-M^-Lac^-$.

The young inocula of the 4 strains were washed carefully and pipetted both singly and in combinations onto plates of minimal agar in dilutions of 1–10 and 1–1,000,000. The total inoculum in each case was suspended in 1 ml. of saline which left a film of moisture on top of the agar in which the organisms could move freely. Some of the seeded plates were exposed to ultra-violet light for the periods of time indicated in table 2. Then all plates were placed at 37°C. for two hours at which time some filaments were formed in the plates irradiated for the longest period. At that time another layer of minimal agar was poured on the plates of the 1–10 dilution and nutrient agar was poured on the plates containing the 1–1,000,000 dilution. After incubation the latter plates gave the total numbers of organisms involved and the former gave a measure of the number of recombinations which had occurred. It will be observed that the maximum production of prototrophs occurs with about 10 seconds exposure to ultra-violet under the conditions of our experiment but that even two seconds exposure enhanced the rate of occurrence markedly.

TABLE 2

EFFECT OF TIME OF IRRADIATION OF SEEDED PLATES ON RECOMBINATIONS

ORGANISMS	PROTOTROPHS PER MILLION CELLS ON PLATES IRRADIATED FOR (SECONDS): 0	2	5	10	30
58-161 + Y53	0.45	2.5	4.4	5.6	3.2
Y53 + Y87	0.8	3.2	4.1	6.8	7.3
58-161 + Y10	0	0	0	0	0
58-161 + Y87	0	0	0	0	0
58-161	0	0	0	0	0

From a number of plates, colonies were isolated and transferred to eosin-methylene blue agar to determine lactose fermentation. Of the 25 organisms tested from the prototrophs obtained from the combination of two lactose negative strains, no lactose positive organisms were found from either the normal or the irradiated inoculum. From the combination of a lactose positive with a lactose negative strain 28% of the prototrophs were lactose positive from both the control and the irradiated inoculum. These data strongly suggest that the small amounts of irradiation stimulate a sexual mechanism in bacteria as measured by the increased rate of recombination. Whether this stimulates a sexual conjugation or the "crossing-over" after such conjugation, or merely the production of a transforming principle is not evident from these experiments.

TABLE 3

EFFECT OF IRRADIATION ON THE ABILITY OF PROTOTROPHS TO FERMENT LACTOSE

	IRRADIATED INOCULUM			CONTROL INOCULUM		
STRAIN	POSITIVE	NEGATIVE	% POSITIVE	POSITIVE	NEGATIVE	% POSITIVE
58-161 + Y53	7	18	28%	7	18	28%
Y53 + Y87	0	25	0	0	27	0

Two points are of particular interest. The first is the independent confirmation of Lederberg and Tatum's demonstration of regular recombination in *E. coli*, K12 (see Lederberg[4]). The second is the marked increase in rate of recombination due to the irradiation. The reasons for this increase are unknown, but the fact suggests an interesting adaption, perhaps connected with the abnormal growth pattern of the irradiated cells.

[1] Gates, F. L., "The Reaction of Individual Bacteria to Irradiation with Ultraviolet Light," *Science*, **77**, 350 (1933).

[2] Robinow, C. F., "Cytological Observations on *Bact. coli*, *Proteus vulgaris* and Various Aerobic Spore-Forming Bacteria with Special Reference to the Nuclear Structures," *J. Hyg. Camb.*, **43**, 413–423 (1944).

[3] Dienes, L., *Proc. Soc. Exper. Biol. Med.*, **66**, 314–317 (1947).

[4] Lederberg, J., "Gene Recombination and Linked Segregations in *Escherichia coli*," *Genetics*, **32**, 505–525 (1947).

*STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. VIII. PRODUCTION OF A GRISEIN-LIKE ANTIBIOTIC BY A STRAIN OF STREPTOMYCES GRISEUS**

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Since the recent demonstration[1] that certain antibiotic agents produced by actinomycetes possess bacteriostatic and bactericidal properties against *Mycobacterium tuberculosis*, a considerable interest has arisen in a systematic study of similar potentialities among the practically unlimited strains and species of actinomycetes that could be isolated from various natural substrates.[2, 3]

The present investigations were initiated to determine the presence in the feces of healthy and tuberculous herbivorous animals of actinomycetes which have growth-inhibiting properties against mycobacteria and especially *M. tuberculosis*, and of the production by such organisms of antibiotics which have similar properties.

A culture of an organism belonging to the *Streptomyces* was isolated from the fresh stool of a healthy heifer, and found to be highly effective. This culture (No. 3510) was tested against four mycobacteria, namely, *M. ranae*, *M. avium*, *M. phlei* and *M. tuberculosis* 607, a fast-growing non-pathogenic strain of the human tubercle bacillus. These tests were made by the agar cross-streak method[4] on three different media: dextrose-asparagine agar, nutrient agar and egg albumin agar. A streptomycin-producing strain of *S. griseus* was included among the organisms for comparison with the more active unknown cultures.