# THE EFFECTS OF DNA AND ENZYME-TREATED DNA ON BACTERIAL POPULATION CHANGES

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Desoxyribose nucleic acid (DNA) is suspected of a fundamental role in controlling specific biological activities, which includes its implied genetic function as a component of the chromosomal system and its ability to cause specific transformations of the genotype in various bacterial species.[1] In view of such implications, any demonstration of less specific effects of DNA upon changes in the genotypic composition of a cell population becomes of interest. Such less specific effects of DNA were observed in recent studies with Brucella populations which attempted to determine the possible existence of phenomena akin to transformation among Brucella strains.

DNA was isolated from Brucella cells by two procedures: (*a*) by extracting cells, treated with desoxycholate, according to the procedure of McCarty and Avery,[2] and (*b*) by a similar procedure (deproteinization and alcohol precipitation) applied to the supernatant of heavy cell suspensions in 0.5% phenol, which had been shaken for 48 hours at 37°C. in the presence of glass beads. The activity of the two preparations was equal, but the yields of highly purified DNA, identified as such by physical and chemical methods (ultra-violet absorption, solubility, Dische-Stumpf reaction, hydrolysis and chromatography), were far greater by procedure (*b*). Population changes in liquid media, in the presence or absence of DNA preparations, were determined by spreading a sample of the culture on agar where the phenotypes (smooth or *S*, rough or *R*, mucoid or *M* morphology) of the resulting colonies indicated the frequency in the population of cells with altered genotype.[3] Early results with a number of *B. suis*, *B. abortus*, and *B. melitensis* strains indicated that initially homogeneous broth cultures supplemented with heterologous DNA tended to show slightly enhanced population changes involving the establishment of a variety of colonial types, including mutants resembling the type from which the DNA had been obtained. In contrast, addition of homologous DNA tended to suppress population changes. In order to test the dependence of these effects upon DNA, desoxyribonuclease (DNase) was added to cultures that had been supplemented with either heterologous or homologous DNA. Under such conditions certain strains exhibited very extensive population changes, whereas control cultures without DNA + DNase, without DNA, or without DNase, failed to exhibit changes of comparable extent (for example, see table 1). Since these striking population changes occurred even after addition of DNase to cultures containing homologous DNA, it was suspected that the effects of enzyme-treated DNA and the above-cited effects of heterologous DNA alone, might involve different mechanisms. The effects produced by enzyme-treated, presumably depolymerized DNA, therefore were investigated more thoroughly and the results obtained in such studies represent the major subject of this communication.

*B. abortus*, Strain 19 *M* (mucoid) cells were used as inoculum in all studies on the effects of DNA + DNase, and the establishment of *S* (smooth) cells was determined by streaking samples from aging beef-extract broth cultures on 2-1 agar.[3] In

most experiments supplementation with 150 $\gamma$ DNA and 75 $\gamma$ DNase per ml. was employed. Table 1 presents a typical result, showing the high degree of population change occurring in cultures supplemented with DNA + DNase. Experiments in which the DNase was inactivated, prior to addition to the culture medium, by brief boiling of the DNA + DNase mixture after various intervals following the addition of DNase, revealed that a very brief exposure to DNase (less than 5 seconds) sufficed to produce the sufficiently altered state of DNA responsible for the effect. Control experiments showed that similar boiling of DNA alone produced no significant effects. Purines and pyrimidines (xanthine, hypoxanthine, guanine, adenine, uracil, thymine), as well as acid hydrolysates of bacterial DNA, were found entirely ineffective in duplicating the effects of presumably slightly depolymerized

TABLE 1

THE EFFECTS OF DNA, DNase, AND DNA + DNase UPON POPULATION CHANGES OF INITIALLY *M* CULTURES OF BRUCELLA ABORTUS GROWN IN BUFFERED BEEF-EXTRACT BROTH

| MEDIUM | EXTENT OF POPULATION CHANGES AFTER: 3 DAYS | 5 DAYS | 9 DAYS |
|---|---|---|---|
| Control | <0.001% *S* | <1% *S* | <1% *S* |
| DNase | <0.01% *S* | 2% *S* | 4% *S* |
| DNA | <0.01% *S* | 3% *S* | 6% *S* |
| DNA + DNase | <1% *S* | 33% *S* | 60% *S* |

TABLE 2

RESULTS OF A TEST ON THE EFFECTS OF DNA, DNase, AND DNA + DNase UPON MUTATION RATES FROM STREPTOMYCIN SUSCEPTIBILITY TO STREPTOMYCIN RESISTANCE

Approximately 5 × $10^9$ cells treated for 6 hrs. at 37°C., and appropriate controls, were plated on plain agar, covered by a protective layer of soft (0.7%) agar; a layer of soft streptomycin-containing agar was poured on top either immediately or 12 hrs. later; following application of the streptomycin layer the plates were kept at 5°C. for 12–18 hrs., to permit diffusion of the antibiotic, and were subsequently incubated at 37°C. for 6 days before scoring of the growth of resistant mutants.

| TYPE OF TREATMENT | AVERAGE NUMBER[a] OF RESISTANT COLONIES THAT DEVELOPED WHEN STREPTOMYCIN WAS ADDED AT: 0 HR. | 12 HRS. |
|---|---|---|
| None | 0 | 4 |
| DNA | <1 | 2 |
| DNase | <1 | 6 |
| DNA + DNase | 5 | 5 |

[a] Average of triplicate tests.

DNA. The effect is not specific for Brucella DNA, isolated from various smooth and nonsmooth strains, but was also obtained in Brucella cultures to which *E. coli* DNA (obtained by procedure (*b*) above) + DNase had been added. Highly polymerized commercial DNA (Worthington), similarly exposed to DNase, however, showed little activity. A number of appropriate tests, involving the determination of mutation rates from $M \rightarrow S$ as well as from streptomycin susceptibility to streptomycin resistance, demonstrated that the enzyme-treated DNA, affecting population changes, does not possess any obvious mutagenic activity. However, these tests appear to confirm previous observations with *E. coli*[4] in regard to a shortening of phenotypic lag in the presence of nucleic acid (see table 2). This shortened phenotypic lag did not suffice to explain the observed effects of DNA + DNase

upon population changes. Additional mechanisms responsible for the non-specific effect of presumably partly depolymerized DNA upon enhancing population changes were elucidated by a more thorough examination of the growth of *S* and *M* cells by themselves, or in mixture, in the presence or absence of DNA + DNase. As figure 1 illustrates, it was found that the presence of DNA + DNase does not modify the growth of *M* or *S* cells when they are by themselves. However, a pronounced inhibition of the growth of *M* cells, even subsequent "killing" of such cells, occurs in the presence of DNA + DNase when *S* cells are present, thus endowing the latter cells with a high selective value in mixed *M* + *S*, DNA + DNase-containing cultures. Furthermore, it was observed that the initial addition of more than 1 per cent *S* cells to *M* cultures promotes the gradual but relatively slow es-

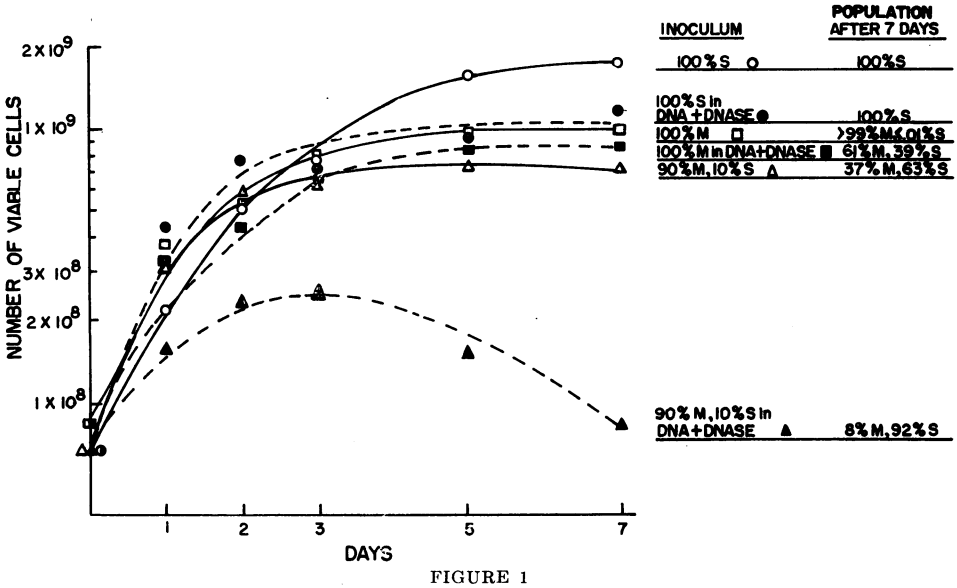


FIGURE 1

Growth curves (viable cell counts) of initially *M*, *S*, or *M* + *S* cultures of *Brucella abortus* in the absence (——) and presence (– – –) of DNA + DNase. Wherever population changes occurred, i.e., in initially *M* and *M* + *S* cultures, the graph depicts the number of *M* cells only. (The count of *S* cells, growing in the presence of *M* cells, increased in the same way regardless of whether DNA + enzyme were added or not.)

tablishment of *S* cells even in the absence of DNA + DNase; in contrast, when such *S* cells are present initially in a proportion of less than 1 per cent they never establish themselves in aging cultures. Also, transfer of cultures at different intervals from media containing DNA + DNase to plain media will enhance the subsequent extent of *M*→*S* population changes in comparison to control cultures, but the rate of changes in such transferred cultures is less than in parallel cultures containing DNA + DNase. On the basis of these observations at least three factors may be held responsible for enhanced population changes in the presence of DNA + DNase: (1) shortening of the phenomic lag leading to the earlier establishment of phenotypically and genotypically altered cells; (2) a significant inhibitory effect upon the parent cells in the presence of a certain number of mutant cells

whenever enzyme-treated DNA is present; and (3) an additional ability of mutant cells to attain a positive selective value as soon as they are present in sufficient proportions, an effect which, as indicated by the "initial mixture" and "transfer" experiments, may be quite independent of the presence of the DNA "derivative." The nature of the inhibitory factor(s) produced by *S* cells in the presence of DNA + DNase is unknown, but may involve a metabolite since the effectiveness of the DNA + DNase effect depends not only upon the strain but also upon the type of medium employed. Finally, the ability of DNase alone to enhance population changes slightly (table 1) may indicate a normal accumulation of DNA in the culture medium which is subsequently converted into "active" DNA by the presence of DNase.

It remains to be determined whether the described non-specific effects of a DNA-like substance upon bacterial population changes might play a secondary role in the more specific phenomenon of bacterial transformations caused by highly polymerized DNA. A more comprehensive report of these studies will be published elsewhere.

[1] Austrian, R., *Bact. Rev.*, **16**, 31 (1952).
[2] McCarty, M., and Avery, O. T., *J. Exptl. Med.*, **83**, 105 (1946).
[3] Braun, W., *Am. Naturalist*, **86**, 355 (1952).
[4] Witkin. E., and Flint, J., in *Carnegie Institution Year Book*, **48**, 161 (1949).

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## *THE PRODUCTION OF MUTATIONS IN DROSOPHILA MELANOGASTER BY THE FAST NEUTRON RADIATION OF AN ATOMIC EXPLOSION**

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Recently the United States Atomic Energy Commission gave us the unusual opportunity of making a small scale study of the effects of the high intensity fast neutrons produced by an atomic explosion on the mutation rate in the mature sperm of *Drosophila melanogaster*. The males were shielded during the explosion so as to avoid temperature extremes, blast, and nearly all of the other radiation produced by the explosion. Dosimeters were included with the flies, and the estimated dosage received by the flies was calculated and given to us in terms of Roentgens Equivalent Physical (REP).

In the laboratory within a week after exposure the radiated males were mated individually in part to Muller-5 females and in part to *res* females, *res* being a combination of eight recessive third chromosome phenotypic mutations used in a similar test of x-rays by Alexander.[1] The offspring of the Muller-5 cross were used for sex-linked lethal tests by Levine. The offspring of the *res* cross were classed by Ives for four groups of visible mutations: *res*-like (resembling one or another of the eight mutants of that stock), Minute (thin bristles), other bilateral mutants, and unilateral mutants of all kinds. The recovered sex-linked lethals were tested by