

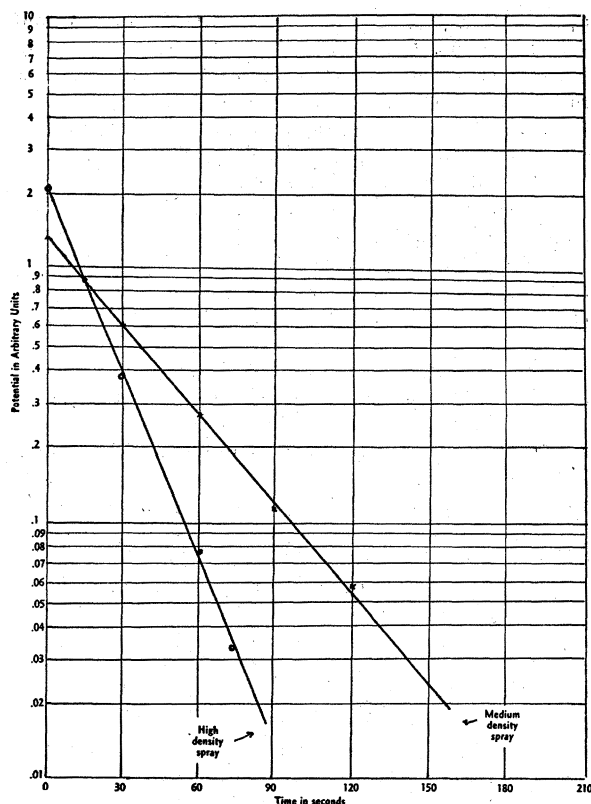


FIG. 5. Medium- and high-density charcoal sprays on polyethylene positively charged.

sity spray experiments were done using charcoal, and again the exponential type decay was obtained; however, the half-time decays were of an order of magnitude faster than in the case of the low-density spray clouds, and are shown in Table 1 and Figs. 4 and 5. Repeated tests with powders gave results reproducible to within 10%.

In both liquid and solid sprays an exponential-type decay of the charge on the polyethylene plate is obtained, and its rate of decay is dependent upon the density of spray and material used. This serves to indicate the net polarity and the magnitude of the spray cloud. In the case of the spray deposit of 1:1 charcoal-starch mixture, it is found that it produces a considerably faster half-time decay on the charged polyethylene plate than in the case of the individual powders. If the charging of the solid particles is assumed to be caused by triboelectric phenomenon—i.e., contact of particles with metal—then it would appear that the phenomenon operates at greater efficiency in the case of the mixture. Since, after spraying has begun, some of the particles adhere to the nozzle, the available contact surface is decreased, thereby decreasing the opportunity for the triboelectric phenomenon to operate. In the case of a mixture, however, both types of particles would adhere to the nozzle, permitting the triboelectric phenomenon to operate more efficiently. It would seem that the data qualitatively verify this. No satisfactory explanation

seems available for the change in the ratio of positive to negative carriers for charcoal sprays when the density is increased.

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Prevalence of *Escherichia coli* Strains Exhibiting Genetic Recombination

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The first bacterium to be tested by an efficient selective method for the occurrence of genetic recombination was strain K-12 of *Escherichia coli*. Experiments with auxotroph mutants of this strain promptly gave conclusive, positive evidence of genetic exchanges between different mutant cells in mixed cultures (1, 2). However, subsequent attempts to obtain comparable results with a number of other strains used for genetic work were fruitless.

Cavalli and Heslot (3) examined a number of auxotroph strains from the National Type Culture Collection (England) and found one that could be crossed with K-12. Unfortunately, this isolate has a complex nutrition, so far unanalyzed, which greatly hinders further work. In other characteristics it closely resembles K-12.

It would be surprising if K-12, the first *E. coli* strain examined, should prove to be uniquely suitable for crossing experiments. Unfortunately, the method for testing fertility involved a good deal of work: it was necessary to prepare at least two nonoverlapping, double nutritional mutants from each strain. Despite improved techniques (4), such a procedure is almost prohibitive for routine survey of new strains. The following procedure was therefore put into effect for preliminary screening.

A multiple marker strain, W-1177 (= 677-str in [5]) has been developed from K-12 by a long sequence of mutational steps. This strain differs from the wild-type strain K-12 in these markers: polyauxotrophy for threonine, leucine, thiamin; resistance to streptomycin and to bacteriophage T1; failure to ferment lactose, maltose, mannitol, xylose, galactose, or L-arabinose. These may be symbolized as: $T-L-B_1-S^r V_1^r Lac-Mal-$ etc. Typical wild-type *E. coli* strains are $T+L+B_1+S^r$. These four markers are useful in detecting recombination between W-1177 and new strains to be screened. Heavy inocula of W-1177 and

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of the propositus are mixed in a complete broth tube, and incubated for 6–24 hr. The mixed culture is then harvested, and the washed cells are plated on a minimal agar medium containing 100–1,000 µg/ml streptomycin. The minimal agar selects prototroph cells; the streptomycin selects S^r . The minimal streptomycin agar thus permits the growth only of $T + L + B_1 + S^r$ colonies and suppresses the two parents. This assortment of characters can arise either by recombination, or by mutation of the propositus from S^s to S^r . Fortunately, this mutation occurs at an extremely low rate, about once per 10^{10} cell divisions (6), and therefore confusion between recombinants and mutants is minimized. On the other hand, the improbable coincidence of three reverse mutations needed to produce a prototroph from W-1177 has never been observed in extensive controls (1, 2).

The principal function of the screening procedure is the rational selection of cultures appropriate for more detailed analysis by the development of auxotroph mutants. Even in this preliminary test, however, recombination of unselected markers (V_1 , Lac , Mal , etc.) among the S^r prototroph selections usually verified the occurrence of genetic interchange.

Two groups of cultures have been screened so far for cross-fertility with W-1177 (i.e., K-12). About 40 cultures from chicken cecal flora (supplied by courtesy of S. Shapiro) yielded one isolate that crosses, but very poorly. About 100 isolations from human urine cultures (secured through courtesy of the Wisconsin State Laboratory of Hygiene) have given 8 that cross with about the same facility as K-12, and an equal number that appear to be less fertile (if fertile at all), so that the evidence for recombination in the latter is still inconclusive. The possibility that some ecotypic differentiation is revealed by the breeding test deserves further study when it is recalled that K-12 is also of human origin.

Nutritional mutants are being prepared in the new isolates. The three cultures so far tested cross freely with each other, as well as with K-12 and within each strain.

The new strains differ in a number of characteristics, including fermentation patterns (3 are sucrose-positive; 6, sucrose negative; one is a lactose-negative "paracolon" type), colony morphology (R , S , and intermediates by the acriflavine test), and patterns of resistance to and production of colicins (7) and phages. Preliminary serological studies are under way, in addition to experiments to uncover cryptic genetic differentiation. There is a strong suggestion that colicin and lysogenicity interactions may act as genetic isolation mechanisms.

Unfortunately, the survey method does not reveal other intrafertile, intersterile breeding groups, nor, owing to the dominance of S^s (8), can it reveal unreduced diploid hybrids between the different strains. Despite these shortcomings, however, the streptomycin-prototrophy selection method has succeeded in displacing strain K-12 from its position as the only "sexual" bacterium.

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The Mode of Action of Growth Substances and Growth Inhibitors

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Previous experiments have proved (1) that alcoholic extracts of different plants show various effects in regard to elongation of cells. Interpreting these results, it is reasonable to suppose there are growth-promoting and growth-inhibiting substances in the plants. Amounts of the two types of growth regulators vary in different kinds of plants. Extracts of *Brassica* sp. show a strong growth-promoting effect, whereas extracts of *Syringa* produce a strong growth-inhibiting effect.

It was therefore of interest to study the effect of a mixture of growth substances and inhibitors by means of the *Avena* test. Larsen (2) used the Went test, taking the angle of curvature for a criterion of the effect, but his results are not very clear and are difficult to survey. Hence the question was studied again by means of Linser's (3) paste method, permitting measurement of the coleoptyl elongation (Z) and the angle of curvature (α°).

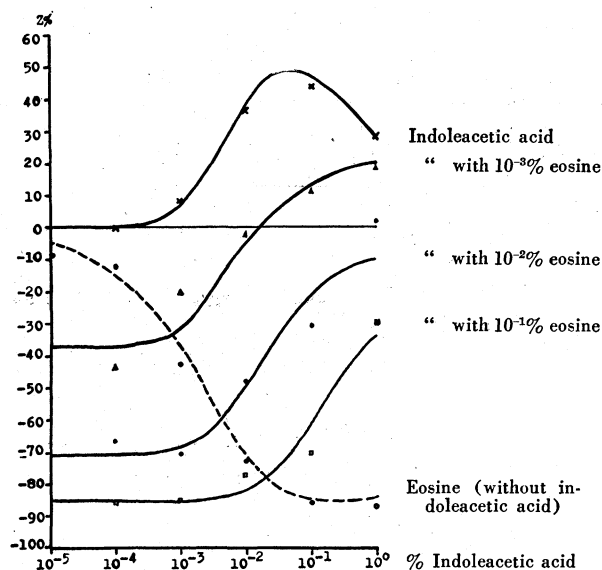


FIG. 1. Mixtures of indoleacetic acid and eosine in different concentrations. The solid lines show the curves calculated for the different mixtures of indoleacetic acid and eosine. On the left, the curve of action is calculated for eosine. The corresponding experimental values are marked as single points.