Bacterial Genetics and Drug Resistance

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The emergence of drug-resistant organisms in patients receiving chemotherapy has presented a serious medical and epidemiological problem. To a certain extent this problem is abating; for although most organisms readily develop significant degrees of resistance to sulfonamides or streptomycin, there has been little clinical appearance of resistance to penicillin, except among staphylococci, or to the newer antibiotics (chloramphenicol, aureomycin, and terramycin). In the treatment of tuberculosis with streptomycin, however, drug resistance remains a major limitation.

This problem in tuberculosis has been discussed in detail by Yegian and Vanderlinde (*1*). The present paper will particularly consider drug resistance in the light of developments in bacterial genetics.

Bacterial Variations

In this recently expanding discipline, bacterial variations, long studied by bacteriologists in an empirical manner, have been re-examined from the point of view of modern genetics (*2*). With few exceptions, these variations have been found to fall into two classes: physiological and genetic. Physiological adaptations to a changed environment involve all the cells in a culture, and are noninheritable, being reversed by return to the original environment; genetic changes, in contrast, involve only a tiny fraction of the cells in the original population, and are inheritable, being transmitted from generation to generation of the offspring of the changed cells, even when grown in the original environment. Drug resistance belongs to the inheritable class, which also includes inheritable changes in a variety of other characteristics, such as morphology, nutritional requirements, and virulence.

Inheritable bacterial variations resemble the mutations of higher organisms, as Beijerinck pointed out within a few months after the discovery of the latter by De Vries in 1900. Only within the past decade, however, has it become generally recognized that the two processes are alike in several respects: not only are their effects inheritable, but both changes occur spontaneously in an exceedingly small fraction of a population of cells, and both are increased in frequency by certain physical agents (ultraviolet, X, or radioactive irradiation) or certain chemicals (e. g., nitrogen mustards). The resemblance is further emphasized by recent evidence that bacteria have much the same genetic apparatus as do cells of higher forms: nuclei have been demonstrated in bacteria (*3*), and within these nuclei there are chromosomes which appear to undergo mitosis (*4*). Furthermore, some bacterial strains can inherit features (including acquired drug resistance) from two different parents, as in the sexual process of higher organisms (*5*). Let us, therefore, briefly consider the nature of genetic mutations. A stimulating exposition of genetic principles can be found in Schrödinger (*6*).

Mutations

By many lines of evidence it has been shown that almost all the inherited properties of ani-

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mals or plants are transmitted by their genes—self-duplicating material units or regions located in chromosomes in the nuclei. The inborn nature of an organism is determined by the combination of genes it receives from its parents. Usually each individual gene is transmitted unchanged from generation to generation, but rarely—with most genes, once in a million to a billion cell generations—a gene will spontaneously undergo an inheritable change. These changes are called mutations; a cell or organism bearing a mutated gene is called a mutant. Mutations occur at random; it is impossible to predict which individual in a population, or which gene in an individual, will undergo mutation in a given generation.

These spontaneous mutations, along with Darwinian natural selection, are considered by modern geneticists to be the mechanism of evolution in the biological kingdom. Mutations of all sorts are constantly occurring in all species; each environment selects for survival those mutants that are especially fitted for that environment. Even in the nineteenth century, before the discovery of mutations, biologists had largely abandoned the alternative Lamarckian view that organisms can inherit characteristics acquired by a specific useful or purposive adaptation to the environment. All experimental attempts to demonstrate such a process failed.

It should be emphasized, however, that the nineteenth-century experiments that defeated Lamarckism involved only characteristics (e. g., mutilations) acquired by somatic cells. And, indeed, one could not conceive, in terms of modern biology, that a giraffe's neck stretched or a puppy's tail hacked by an experimenter could lead to a longer neck or a shorter tail in the next generation. The hereditary nature of this generation would be determined by the parents' germ cells—the spermatozoa and ova—and these have no evident means of reflecting a mechanical change in the somatic cells.

But is it safe to exclude the Lamarckian theory for one-celled species as well as for higher organisms? In bacteria there is no distinction between germ cell and somatic cell; there are only genetic (heredity-determining) and nongenetic parts of a single cell. It is therefore conceivable that a drug, having penetrated into a bacterial cell, might somehow direct changes in the genetic part of the cell that would result in a mutation to drug resistance. To be sure, the fact that resistance arises only in a tiny fraction of the population might seem to suggest a spontaneous origin of the mutation, but it hardly proves such an origin—for the very test for the presence of these resistant cells in the population always requires exposure of the population to the drug, and it might be *during* that exposure that the mutation first occurs. Neither the nineteenth-century polemics on evolution nor careful scrutiny of the ordinary drug experiments can settle the issue; a subtler approach is needed.

Evidence for Spontaneous Mutations

The question was answered definitely by a statistical approach (fluctuation analysis) designed by Luria and Delbrück (*7*) to study the similar problem presented by bacteriophage resistance and subsequently applied by Demerec (*8*) to drug resistance. The argument runs as follows: Let a few colon bacilli, including no drug-resistant mutants, be inoculated into a flask containing 100 ml. of medium, and at the same time inoculate a few bacilli into 100 tubes each containing 1 ml. of medium. After incubation, the total number of bacteria in the 100 small vessels is the same as the number in the large one. And if the contents of the 100 small vessels are mixed, and samples from this mixture and from the original flask are tested by plating in the presence of the drug, the number of resistant mutants is also found to be about the same in the two lots of bacteria.

But if the 100 tubes, instead of being mixed, are separately tested, what will the distribution be? There are two possibilities. If the mutations do not occur until exposure to the drug, these separately grown samples should be indistinguishable from 100 samples from the single flask and should show the same distribution as is found with the latter, namely, a constant number of mutants in each sample, except for the inevitable statistical variation in sampling. But if the mutations have occurred before the test with the drug, the numbers of mutants in the 100 tubes should fluctuate more widely, for the following reasons. Since mutations are chance events, some tubes will develop a first

mutant earlier than others. And since mutants breed true, each mutated cell will give rise to a family of mutants that doubles with each generation as long as the population continues to grow. Hence, the tubes with an early mutation will have a large number of mutants when full growth is reached while, at the other extreme, some tubes will have few or no mutants. The question, then, is simple: Will the fluctuation in the number of mutants be the same in the separate tubes as in the samples from the single flask?

When the experiment was performed, the fluctuation was found to be much greater with the 100 separately grown tubes than with 100 samples from a single culture; indeed, a few "jackpot" tubes yielded hundreds of mutants while others yielded none. In these jackpot tubes the first mutation must have occurred many generations before the culture stopped growing. The issue, then, has been critically settled in favor of the classical genetic view: Mutations to drug resistance have occurred before exposure to the drug, and the drug then acts as a selective agent.

Fluctuation analysis shows how an ingeniously designed experiment can furnish a decisive conclusion that replaces earlier opinions formed on the basis of intuitions and analogies—often the only available basis for answering complex medical and biological questions, but hardly a satisfying one. Even more direct evidence for spontaneous origin of drug-resistant mutants has since been obtained by other workers (Newcombe, Lederberg) who spread bacteria densely on a plate of medium without a drug and showed that after some growth resistant cells, detected by subsequent transfer, were present in clusters.

Some investigators still believe that drugs play a directive role, but their evidence does not include the critical test of fluctuation analysis. The present state of the problem can be summed up by saying that spontaneous mutation plus selection has been demonstrated in some cases of drug resistance; drug-directed mutation has been demonstrated in no case; but drug-directed mutations are still theoretically possible.

There is little doubt that spontaneous mutation to drug resistance occurs in the patient at much the same rate as that at which it occurs in the test tube. In either circumstance, however, the speed of emergence of a predominantly drug-resistant population depends not only on the rate of mutation but also on the efficiency of selection. In the homogenous environment of the test tube, the possibility of sharp selection, with survival or proliferation only of mutants resistant to a given concentration of drug, allows precise quantitative experiments. In the experimental animal or patient, however, selection will be affected by a variety of other factors. These include variations, with respect to time and to region of the body, in drug concentration, bacterial population density, rate of multiplication, rate of bactericidal action, and in the elimination of mutants and nonmutants by host defenses. It is easy to see how these complicated interactions of drug, parasite, and host can reduce the predictability of the results. But there is little likelihood that the host can alter the primary process of spontaneous mutation.

Persistence of Resistant Strains

What happens to drug-resistant strains after the drug is eliminated from their host, or when they are cultivated in drug-free media? It is known that most mutants revert at a low rate, by a second mutation, to strains that behave like the original parent. But these reverted strains will not replace the predominant strain unless they have some selective advantage over the latter. Some resistant mutants do grow more slowly than their parents or their reversions, and hence tend gradually to be outgrown by sensitive reversions when cultivated in the absence of the drug. But other resistant mutants are just as fast-growing, and just as virulent, as their reversions, and hence tend to persist in the population.

This persistence in the population has important epidemiological consequences. The widespread use of a drug among the hosts will alter the ecology of a parasite, and hence may lead by a selective process to widespread distribution of strains resistant to that drug. A striking example is presented by gonorrhea, a disease that offers an especially favorable opportunity for such selection since its transmis-

sion is restricted to human beings. In New York City, for example, the proportion of cures achieved with sulfonamides dropped from about 90 percent in 1936 to about 30 percent in 1942. Had other drugs (e. g., penicillin) not become available, the chemotherapy of gonorrhea would have stopped. At present, a similar spread of penicillin-resistant staphylococci and, to a much smaller extent, of streptomycin-resistant tubercle bacilli, appears to be taking place.

Approach to Drug Resistance

How can a knowledge of the genetics of drug resistance help the physician and the public health worker? For one thing, it tells us that, though mutation rates can be increased by a variety of physical and chemical methods, no method for decreasing these rates is known or seems likely to appear. The best prophylactic approach to drug resistance, therefore, appears at present to consist of attempting to suppress the selection of mutants, since there is no way of suppressing their formation. And in this search for a method of suppressing selection by a drug, genetics offers positive help. It provides a clear rationale for a method that was originally suggested, on intuitive grounds, by the founder of chemotherapy as early as 1912 (*9*) : combined therapy with two independently acting agents. If one cell in 10^6 mutates to resistance to one drug, and one in 10^6 to another drug, only one in 10^{12} will simultaneously develop both mutations. Hence, doubly resistant mutants have a negligible probability of emerging from a sensitive strain in the presence of effective concentrations of two chemotherapeutics with different modes of action, even though such double mutants can easily be obtained by selection, first with one drug and then with the other.

The principle of combined therapy has recently been applied clinically, with encouraging results, in treating tuberculosis with streptomycin plus either *p*-aminosalicylic acid or a thiosemicarbazone. It seems safe to predict that even more satisfactory results will appear as optimal dosage schedules are worked out and better drugs are provided.

Furthermore, the recent applications of genetic principles described here, together with other valuable applications of genetics to the study of intermediary metabolism and drug action, suggest that this field might well be given more emphasis in the future training of physicians and public health workers.

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