Studies of bacteria with acquired resistance to drugs throw light on the mechanism of the resistance phenomenon.

Genetic Basis of Acquired Drug Resistance

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UNTIL a very few years ago many biologists still questioned whether the hereditary mechanism that had been so effectively analyzed in *Drosophila*, maize, *Datura*, and mice, and found to apply to all the higher organisms, was also operative in lower organisms. Bacteriologists in particular were highly skeptical on this subject, probably because they considered it unlikely that such a simple mechanism, if it existed in bacteria, could have escaped detection in the course of their extensive physiological and clinical studies.

Within the past decade, however, there has been a striking change in the thinking of scientists about heredity in micro-organisms. About 10 years ago geneticists started to work with these organisms, and they developed for their research special methods utilizing a broad background of information derived from studies of higher organisms. Their results have been spectacular.

Hershey (*1*) and Delbrück and Bailey (*2*) in 1946 found that crosses could readily be made between bacterial viruses (bacteriophages), and experiments by Visconti and Delbrück (*3*) in 1953 indicated that the transmission of paternal characteristics to the offspring of such crosses is in accordance with expectation based on Mendel's law. These findings mean that the basic genetic mechanism operating in the lowest and smallest of known living beings is similar to that operating in the higher organisms, and they suggest that the fundamental laws of heredity are general and apply to all life. They do not necessarily mean that the mechanism of heredity must be identical in all details throughout the living world, nor, in particular, that chromosomes, which have been established as the carriers of the determiners of heredity (genes) in higher organisms, must be present in the same form in all organisms. Indeed, we have good reason to believe that such chromosomes do not exist in bacterial viruses. It does seem very probable, however, that the basic threadlike structure of the chromosome, the chromonema, is similar in all organisms, but that the envelope surrounding the chromonemata may be absent in the lowest organisms, such as viruses, and present in a different form in some others.

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Whereas a single chromonema is extremely slender and very probably below the level of optical visibility, the envelope makes up the bulk of a chromosome and is responsible for its characteristic appearance as observed at certain stages in dividing cells.

The discovery that bacterial viruses possess a genetic mechanism similar to that operating in higher organisms would in itself have justified the assumption that such a mechanism is present also in bacteria, for it would be remarkable, indeed, if bacteria were set apart in a class by themselves as far as heredity is concerned. In addition to this indirect evidence, however, there is a considerable body of direct evidence about the genetic mechanism in bacteria, proceeding especially from the genetical work of Lederberg and his collaborators, and from cytological studies by Robinow (*4*) and DeLamater (*5*). Thus, we are now able to say with a fairly high degree of certainty that bacteria are not essentially different from other organisms with respect to the genetic mechanism, that is, that they possess genes and chromosomes.

In the early days of genetics, and in some quarters even recently, the opinion prevailed that biologically unimportant characteristics, such as body color, shape of various organs, and the presence or absence of hairs and bristles, were determined by genes, whereas biologically important and fundamental properties, such as taxonomic differences, fertility, and the functions governing development, were determined by some other, unidentified mechanism. But with the progress of genetic research the number of such "fundamental" properties that can be supposed to depend on some extragenic mechanism has rapidly decreased, and at present there is ample evidence to indicate that genes play a major role in the transmission of almost all hereditary traits.

For example, the results of early studies by Ephrussi and his collaborators indicated that cytoplasmic constituents were responsible for the inheritance of a "petite" colony type in yeast, but later these workers were able to show by ingenious tests that genes also play a decisive role. In this yeast, treatment with euflavine suppresses synthesis of the enzyme, cytochrome oxydase, by eliminating from the cells the specific cytoplasmic components (mitochondria) that presumably produce this enzyme. The rate of multiplication of such cells is affected so that they form small colonies rather than colonies of normal size; and this pattern of growth is transmitted to the offspring. Thus, the character appears to be determined by the cytoplasmic units, mitochondria. Crosses made between these small-colony cells and normal cells from another strain of yeast, however, showed that the synthesis of respiratory enzymes requires the simultaneous presence of a cytoplasmic factor and a dominant nuclear gene. The cytoplasmic factor appears to be dependent on the nucleus in its function although independent of it in its reproduction (*6*).

Origin of Resistance

With this brief introduction, in which I have stressed the likelihood that the same kind of genetic mechanism operates in all living organisms and that it plays an important role in the hereditary transmission of almost all characteristics, I shall now take up the problem of genetic basis of acquired drug resistance. The following are well-established facts: (*a*) Bacterial strains more resistant to a certain drug than the original strain can be isolated, sometimes with great ease; (*b*) the degree of resistance may vary from slight to complete; (*c*) as a rule, the resistance is retained through a large number of transfers; and (*d*), again as a rule, the degree of resistance is not affected by the transfers. Thus, it is demonstrated that bacteria of such resistant strains have acquired a new property, which they transmit to their offspring, in other words, that we are dealing with a heritable characteristic.

With this information available, a geneticist would assume a priori that changes in genes—that is, mutations—are responsible for the origin of resistances, since it seems very improbable that some other mechanism could account for such a clear-cut and comprehensive class of properties. Substantial grounds for this assumption have been provided by experimentation carried out in several laboratories. The combined results have shown clearly: (*a*) that resistance is not induced by the presence of a

drug but appears spontaneously, as one would expect if it is caused by gene mutation (*7–14*); (*b*) that crosses made in studies of resistance to streptomycin (*15–18*), chloramphenicol (*19, 20*), azide (*21*), terramycin (*12*), and furadroxyl (Nelson, quoted in reference 22) have given rise to Mendelian segregation and linkage, as expected in genic inheritance; (*c*) that in transduction experiments streptomycin resistance is transferred in the same manner as other genic characters (*23*); and finally (*d*) that in transformation experiments both penicillin resistance and streptomycin resistance are transferred like other genic characters (*24–26*). Thus, in manner of origin and in behavior in crosses, transduction, and transformation, the characters conferring bacterial resistance to drugs conform to the type of reaction usually observed in studies of gene-determined traits.

Within the past decade another important aspect of genic reaction has been explored. It has been found that not only radiations but also a considerable number of chemical compounds are able to induce mutations in genes, and—the significant point for our consideration here—that in every known instance the effect has been nonspecific; that is, in no case studied so far has a chemical been able to induce mutations only in a certain gene or only in genes affecting one particular property. Therefore, again, it would be very surprising indeed to find that treatment of bacteria with a drug had a specific effect on the gene or genes determining their sensitivity to that drug—a postulate implied by those who claim that bacterial resistance to a drug is induced by the drug itself. Fortunately, no such assumption is necessary to account for the observed fact that resistant bacteria invariably appear after exposure of cells to certain concentrations of a drug. This result is to be expected under circumstances that are well known to students of population genetics, namely, when a strong selection pressure acts on a large, nonhomogeneous population in which many genotypes are represented. A bacterial culture or a bacterial infection is just such a population. Since bacterial cells are very small, a tremendous number of individuals can be present in a relatively small volume. For example, 1 cubic millimeter would hold about 10^9 (a billion) cells of *Escherichia coli*. From what is known about the frequency with which spontaneous mutations to drug resistance occur, it is to be expected that a population of that size would include mutants representing almost all the genes instrumental in resistance, and consequently that some bacteria resistant to any particular drug would be present. Treatment with a drug, then, would inhibit or eliminate the large body of sensitive individuals and thus would make it possible for the few resistant bacteria to multiply freely and produce a strain resistant to the drug used in treatment.

Patterns of Resistance

Studies of resistance to different drugs have shown that in the great majority of cases a high degree of resistance is attained only "stepwise," that is, through the occurrence of several consecutive mutations. From a sensitive strain we can isolate a mutant strain that is only slightly resistant (first-step resistance); another mutation occurring in such a strain will produce an increased degree of resistance (second-step resistance); and so on. With a few drugs, however, it is possible for some first-step mutants to possess a high degree of resistance. Thus, effective resistance either may be built up in several steps, or it may be attained through a single mutational event. The stepwise pattern of resistance development has been called the penicillin pattern, and the single-step pattern, the streptomycin pattern, after the drugs first used in analyzing the patterns (*8*). It is interesting to note that, as a rule, the resistance pattern is specific for the drug, in other words, that the origin of resistance to a certain drug follows the same pattern in all strains and species of bacteria.

Pattern of resistance has a very important bearing on the effectiveness with which physicians can avoid the development of highly resistant strains in clinical practice. The use of drug concentrations high enough to eliminate all first-step resistant organisms will preclude the buildup of high resistance where the stepwise pattern of origin is concerned, but no single treatment will be effective in preventing the origin of highly resistant individuals when one is dealing with the single-step pattern. Fortunately, only a small number of known drugs

elicit this single-step pattern of resistance—streptomycin, isoniazid, sodium *p*-aminosalicylate (PAS), erythromycin, and cinnamycin (*27*); all others are associated with the stepwise pattern.

Theoretically, it should be possible to avoid the development of strains resistant to the single-step pattern drugs by administering these drugs in combination with other drugs which act independently of them. In dealing with the stepwise pattern, also, the clinical use of combinations of drugs should still further reduce the chances for development of resistance. The reasoning on which this expectation is based can be outlined as follows. If the frequency of origin of mutant bacteria resistant to either drug A or drug B is 1 per 10 million (1×10^{-7}), and if mutants resistant to drug A are sensitive to drug B and vice versa, then the expected frequency of bacteria carrying both resistances is 1 per 10^{14}. Since only "double" mutants of this kind could survive appropriate treatment with a combination of drugs A and B, and thus become the progenitors of a resistant strain, the chance that resistant strains will develop is tremendously smaller when a combination of drugs is used in treatment than when a single drug is used. Laboratory experiments, the results of which are consistent with this theoretical expectation, have been reported by Szybalski and Bryson (*28*).

In clinical application of drugs, however, other factors than resistance may play a role in the survival of bacteria. Some cells may survive because they are located where they cannot be reached by a full concentration of the drug; others may be in a physiological state that confers a temporary immunity to its action. Under certain circumstances these factors may favor the selection of resistant mutants and result in the development of resistant strains in spite of treatment that theoretically should prevent such development.

In the present discussion I have emphasized the genetic mechanisms that participate in the origin of bacterial resistance to various drugs and have omitted any consideration of complicating factors, such as synergism, cytoplasmic constituents, and physiological aspects. A full discussion of these problems has been ably presented in a comprehensive review by Bryson and Szybalski (*27*).

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Trichophyton tonsurans Ringworm Spreading

A northeastern Michigan outbreak of ringworm infection caused by *Trichophyton tonsurans* was reported in the *Journal of the Michigan State Medical Society*, June 1955, pp. 687–690 and 727, by Eugene A. Hand, M.D., Saginaw, Mich., and Lucille K. Georg, Ph.D., Public Health Service mycologist.

The disease, previously almost unknown in the area, was reported endemic in both rural and urban sections of Michigan where there is a growing community of persons who have come from endemic areas in Mexico.

The Michigan outbreak is indicative of the spread of the disease on the North American continent, according to the Mycology Unit of the Public Health Service Communicable Disease Center.

Within the past 6 to 8 years, *T. tonsurans* infections of the scalp, glabrous skin, and nails have become endemic in New York and in the southwestern States, especially in Texas and southern California. Scattered cases have been reported from 13 States and Canada. Ringworm infections caused by this endothrix fungus have been common in Mexico and Puerto Rico for many years, whereas *Microsporum audouini*, the common agent of ringworm of the scalp in the United States, is rare in those countries.

Public Health Service mycologists foresee the possibility that *T. tonsurans* ringworm may constitute a health problem in the United States similar to epidemic ringworm caused by *M. audouini*. They point out, however, that the problem will be more difficult because the infections occur in both children and adults. The infections are harder to detect and usually are more refractory to treatment. Roentgen ray epilation is considered the treatment of choice.

The problem of *T. tonsurans* ringworm was previously presented in detail in *Public Health Reports*, January 1952, pp. 53–56.