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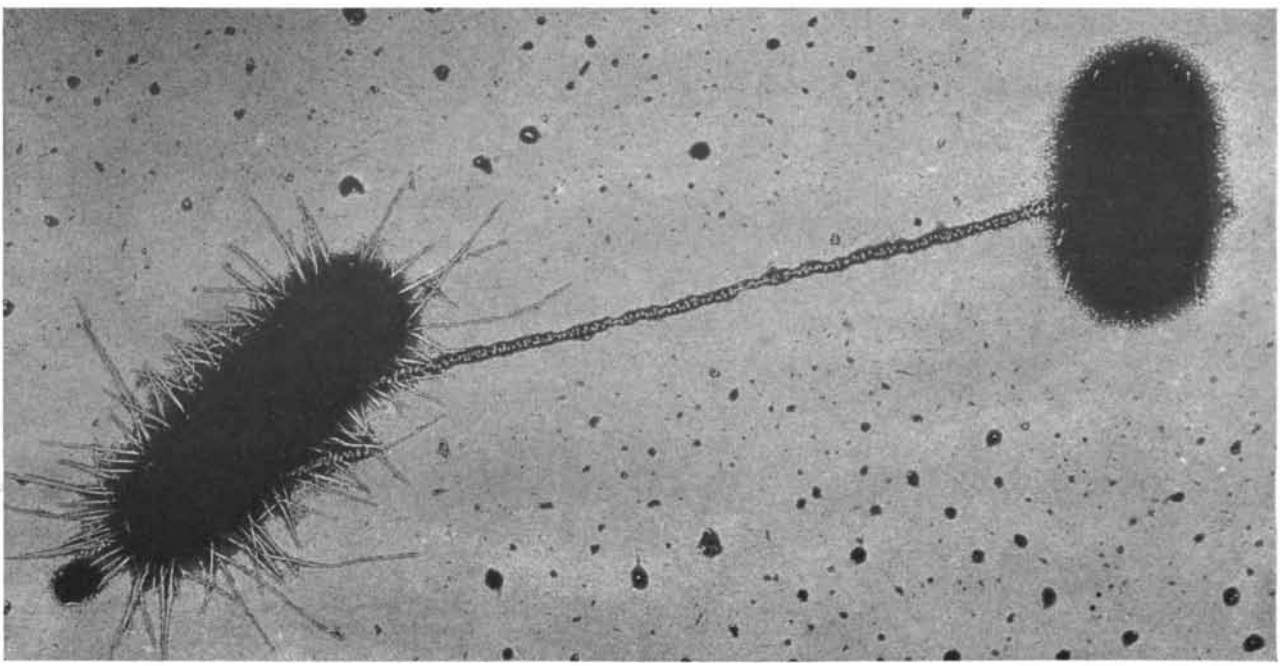
Infectious Drug Resistance

Bacteria can suddenly become resistant to several antibacterial drugs. The resistance is transferred from one strain to another by an "episome" that carries the genes for multiple resistance

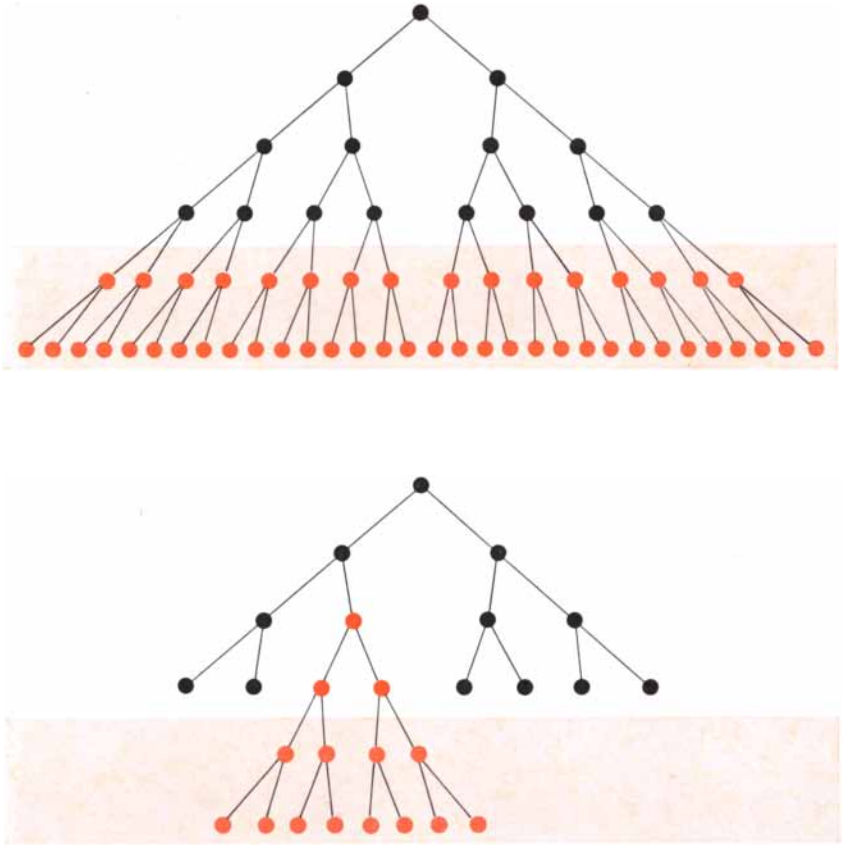
by Tsutomu Watanabe

The advent of sulfonamide drugs and antibiotics brought with it the promise that bacterial disease might be brought under control, but that promise has not been fulfilled. Although many infections respond dramatically to chemotherapy, tuberculosis, dysentery and typhoid fever continue to be endemic in many parts of the world; cholera and plague erupt periodically; staphylococcal infections persist in the most advanced medical centers. One major reason is that the disease organisms have developed resistance to the drugs.

Until recently it was assumed that the appearance of drug-resistant bacteria was the result of a predictable process: the spontaneous mutation of a bacterium to drug resistance and the selective multiplication of the resistant strain in the presence of the drug. In actuality a more



R FACTOR, the particle that imparts infectious drug resistance, is transferred from one bacterial cell to another by conjugation. The various forms of conjugation are thought to be effected by way of thin tubules called pili. In this electron micrograph made by Charles C. Brinton, Jr., and Judith Carnahan of the University of Pittsburgh a male *Escherichia coli* cell (*left*) is connected to a female bacterium of the same species by an *F* pilus, which shows as a thin white line in the negatively stained preparation. Numerous spherical bacterial viruses, or phages, adhere to the *F* pilus. The cells have been magnified about 20,000 diameters.



DRUG RESISTANCE involves a change in the genetic material of a bacterial cell. The change from drug-sensitive cell (*black*) to drug-resistant cell (*color*) is not induced by the presence of the drug (*light color*), as was once thought (*top*). It is the result of a spontaneous mutation that gives rise to cells that survive in the drug environment (*bottom*).

ominous phenomenon is at work. It is called infectious drug resistance, and it is a process whereby the genetic determinants of resistance to a number of drugs are transferred together and at one stroke from a resistant bacterial strain to a bacterial strain, of the same species or a different species, that was previously drug-sensitive, or susceptible to the drug's effect. Infectious drug resistance constitutes a serious threat to public health. Since its discovery in Japan in 1959 it has been detected in many countries. It affects a number of bacteria, including organisms responsible for dysentery, urinary infections, typhoid fever, cholera and plague, and each year it is found to confer resistance to more antibacterial agents. (What may be a related form of transmissible drug resistance has been discovered in staphylococci and may be responsible for "hospital staph" infections.) Quite aside from its importance to medicine, the study of infectious drug resistance is making significant contributions to microbial genetics by illuminating the complex and little understood relations among viruses, genes and the particles called episomes that lie somewhere between them.

If an antibacterial drug is added to a liquid culture of bacteria that are sensitive to the drug, after a while all the cells in the culture are found to be resistant to the drug. Once it was thought that the drug must somehow have induced the resistance. What has actually happened, of course, is that a few cells in the original culture were already resistant; these cells survive and their daughter cells multiply when the sensitive majority of bacteria succumb to the drug [*see illustration above*]. The resistance was not induced by the drug but was the result of a spontaneous mutation. Bacteria, like higher organisms, have chromosomes incorporating the genetic material, and from time to time a gene—perhaps one controlling drug resistance—undergoes a mutation. The mutation of a drug-sensitivity gene occurs only once in 10 million to a billion cell divisions, and when it occurs it alters a cell's sensitivity to one particular drug or perhaps two related drugs.

In 1955 a Japanese woman recently returned from Hong Kong came down with a stubborn case of dysentery. When the causative agent was isolated, it turned out to be a typical dysentery bacillus of the genus *Shigella*. This shigella was unusual, however. It was resistant to four drugs: sulfanilamide and the antibiotics streptomycin, chloramphenicol and tetracycline. In the next few years the incidence of multiply drug-resistant shigellae in Japan increased, and there were a number of epidemics of intractable dysentery.

The familiar process of mutation and selection could not explain either this rapid increase in multiple resistance or a number of other findings concerning the dysentery epidemics. For one thing, during a single outbreak of the disease resistant shigellae were isolated from some patients and sensitive shigellae of exactly the same type from other patients. Even the same patient might yield both sensitive and resistant bacteria of the same type. Moreover, the administration of a single drug, say chloramphenicol, to patients harboring a sensitive organism could cause them to excrete bacteria that were resistant to all four drugs. Then it was found that many of the patients who harbored drug-resistant shigellae also harbored strains of the relatively harmless colon bacillus *Escherichia coli* that were resistant to the four drugs. It was impossible, on the other hand, to obtain multiple resistance in the laboratory by exposing sensitive shigellae or *E. coli* to any single drug; multiply resistant mutants could be obtained only after serial selections with each drug in turn, and these mutants, unlike the ones taken from sick patients, multiplied very slowly.

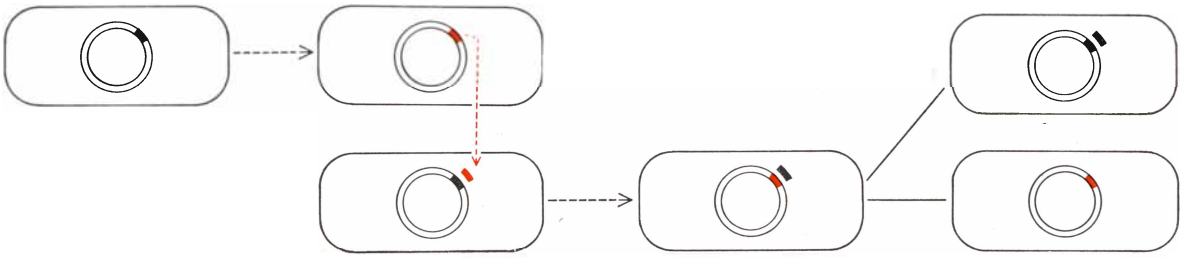
Taken together, these characteristics of the resistant shigellae suggested to Tomoichiro Akiba of Tokyo University in 1959 that resistance to the four drugs might be transferred from multiply resistant *E. coli* to sensitive shigellae within a patient's digestive tract. Akiba's group and a group headed by Kunitaro Ochiai of the Nagoya City Higashi Hospital thereupon confirmed the possibility by transferring resistance from resistant *E. coli* to sensitive shigellae—and from resistant shigellae to sensitive *E. coli*—in liquid cultures. Other investigators demonstrated the same kind of transfer in laboratory animals and eventually in human volunteers. Clearly a new kind of transferable drug resistance had been discovered. What, then, was the mechanism of transfer? There were three known mechanisms of genetic transmis-

sion in bacteria that had to be considered as possibilities.

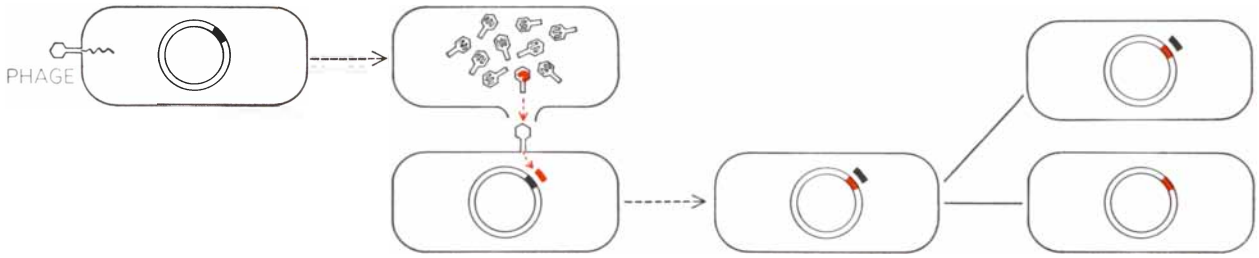
One was transformation, which involves "naked" deoxyribonucleic acid (DNA), the stuff of genes. DNA can be extracted from a donor strain of bacteria and added to a culture of a recipient strain; some of the extracted genes may "recombine," or replace homologous genes on chromosomes of the recipient bacteria, thus transferring a mutation from the donor to the recipient [*see top illustration below*]. In this way, for example, streptomycin-sensitive bacteria can become streptomycin-resistant.

Transformation occurs in a number of different bacteria, and it can occur spontaneously as well as experimentally. Because only small fragments of DNA are taken up by bacteria in transformation, however, it is seldom that more than two different drug-resistance genes are transferred together. It requires optimal laboratory conditions, moreover, for transformation to occur at a significant frequency, and such conditions are not likely to prevail in nature.

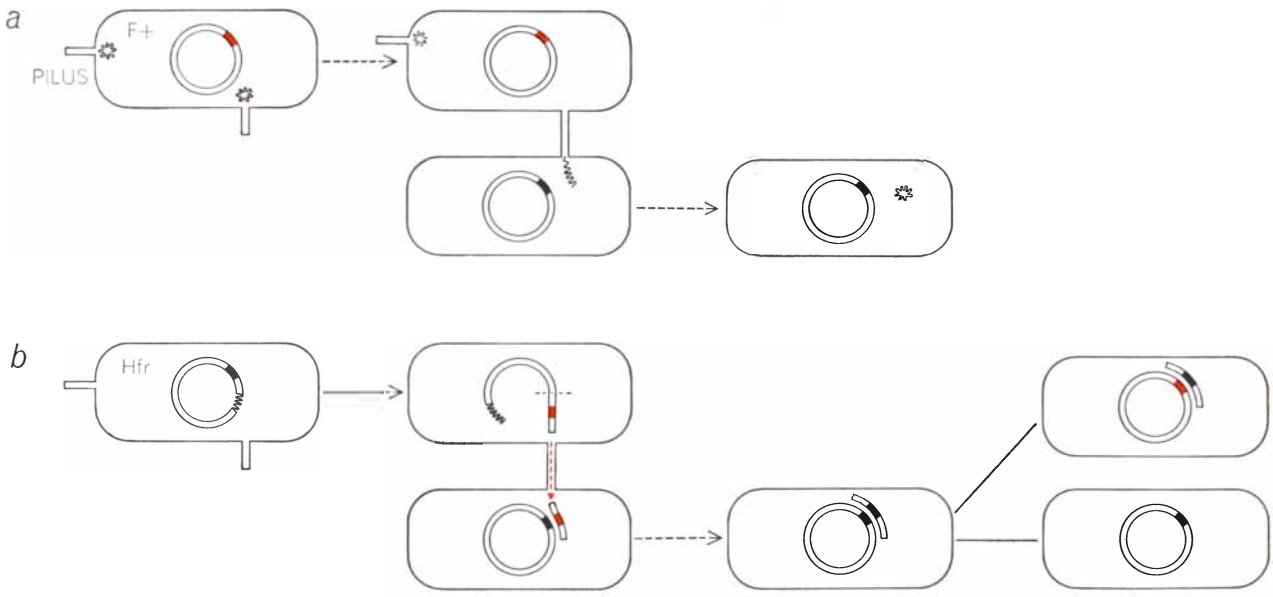
Another mechanism of gene transmission is transduction, in which genes are



TRANSFORMATION is a form of genetic transmission in which deoxyribonucleic acid (DNA) extracted or excreted from a donor cell (*top*) enters a recipient cell (*bottom*) and is incorporated into its chromosome. In this way a mutated gene (*color*) controlling resistance to a drug may be transferred to a drug-sensitive cell, replacing a homologous gene, which is unable to replicate and dies out.



TRANSDUCTION is effected by phage, or bacterial virus. Phage DNA enters a cell (*left*) and directs the synthesis of new phage, killing the cell (*second from left*). A bit of bacterial DNA (*color*), perhaps a mutated gene that causes drug resistance, may be incorporated inside a newly formed phage, be carried to a sensitive cell (*bottom*) and "recombine," or replace a gene on the chromosome.



SEXUAL MATING is a form of conjugation. If a fertility factor (*F*) is in the cytoplasm of a male (F^+) cell (*a*), it is transferred alone through a pilus to a female (F^-) cell. In an *Hfr* cell (*b*) the *F* is incorporated in the chromosome. Cell-to-cell contact causes part or all of the chromosome, perhaps including a mutation for drug resistance (*color*), to pass to a female cell and recombine.

carried from one bacterial cell to another by infecting phages, or bacterial viruses. Transduction occurs when a phage, reproducing inside a cell by taking over the cell's synthesizing machinery, incorporates a bit of the bacterial chromosome within its protein coat "by mistake." When the phage subsequently infects a second cell, the bacterial genes it carries may recombine with homologous genes on the second cell's chromosome. The phage in effect acts as a syringe to bring about what in transformation is accomplished by the movement of naked DNA [*see middle illustration on preceding page*]. Transduction takes place in a variety of bacteria, but at a very low frequency. Genes for resistance can be transduced like other genes, but it is unlikely that more than two resistance genes could be transferred together because the small transducing phage can carry only a short segment of bacterial chromosome.

The third type of genetic transmission in bacteria is conjugation: a direct contact between two cells during which genetic material passes from one cell to the other. Transfer by conjugation occurs primarily from male to female cells of certain groups of bacteria. The male bacteria carry a fertility factor, the *F* factor, that is ordinarily located in the cytoplasm of the cell but may become integrated into the chromosome. When the *F* is cytoplasmic, the male cells are called F^+. In such cells the *F* is readily transferred to female (F^-) cells by conjugation, but it is transferred alone. When the *F* factor is integrated into the bacterial chromosome, it serves to "mobilize" the chromosome. That is, the chromosome, which in bacteria forms a closed loop, opens and portions of it can pass by conjugation to a female cell, recombine with the female chromosome and thereby endow the female bacterium with traits from the male. Because this transfer occurs with a high frequency in male cells with an integrated *F*, such cells are called *Hfr*, for "high frequency of recombination" [*see bottom illustration on preceding page*].

The *F* factor is what is generally called an episome: a genetic element that may or may not be present in a cell, that when present may exist autonomously in the cytoplasm or may be incorporated into the chromosome, and that is neither essential to the cell nor damaging to it. An episome is something like a virus without a coat; indeed, some bacterial viruses can become "temperate" and exist as harmless episomes inside certain bacterial cells [see "Viruses and Genes," by François Jacob and Elie L. Wollman; SCIENTIFIC AMERICAN, June, 1961].

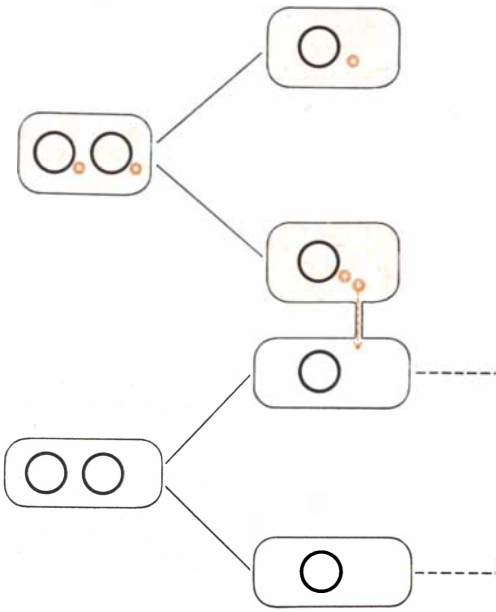
Until recently the actual route of transfer was not known. In 1964 Charles C. Brinton, Jr., of the University of Pittsburgh and his colleagues proposed that the *F* factor or the *F*-mobilized chromosome passes from one cell to the other through a thin tubular appendage, the *F* pilus, that is formed on both F^+ and *Hfr* cells by the presence of the *F* factor. Another kind of pilus, the Type 1 pilus, is seen on female cells as well as male cells, but the two can be distinguished: the *F* pilus is the site of infection by certain phages, and so the phages cluster along the *F* pili, marking them clearly in electron micrographs [*see top and middle illustrations on page 25*].

If a male chromosome transferred to a female cell by conjugation carries drug-resistance genes, these genes may be incorporated into the female chromosome. Experiments with sexual mating showed that drug-resistance genes are in fact sometimes scattered along bacterial chromosomes. Rather long segments—sometimes the entire length—of the chromosome can be transferred in sexual mating, and so it is possible for several resistance genes to be transferred in a single mating event.

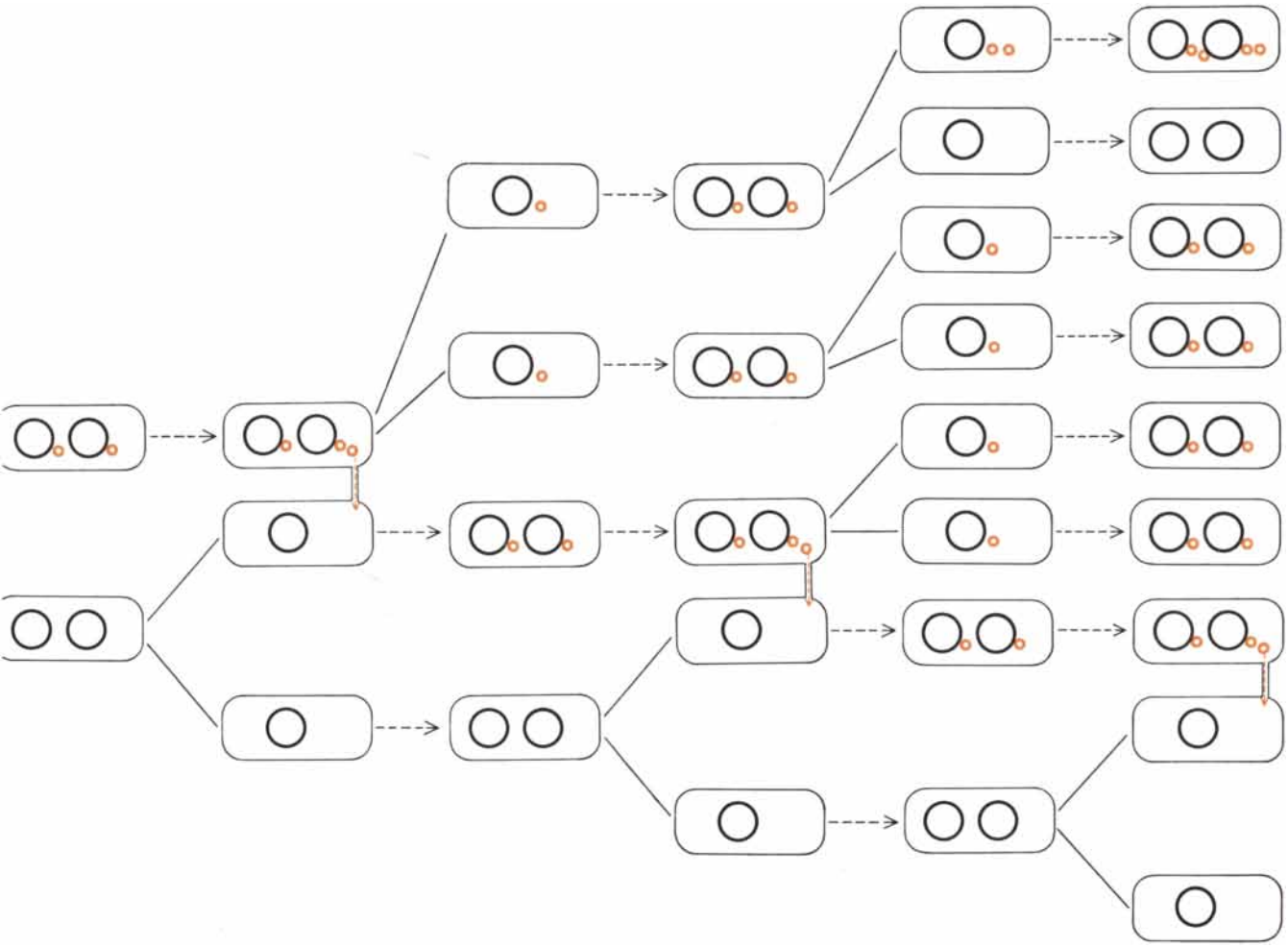
In 1960 we took up the study of the resistant shigellae in my laboratory at the Keio University School of Medicine. It soon became clear that the mechanism of transfer of multiple resistance was not transformation, because sensitive strains were not made resistant by DNA extracted from the resistant bacteria. It was not transduction, because it could not ordinarily be effected by cell-free filtrates of the resistant cultures.

There was strong evidence that some form of conjugation must be responsible. Microscopic examination of a mixed culture of sensitive and resistant bacteria revealed pairing between the different kinds of cells. When a mixed liquid culture was agitated in a blender to break off any cell-to-cell contact, and the culture was then diluted to prevent further pairing, the transfer of resistance ceased. If the mechanism of resistance transfer was conjugation, however, it was not the familiar process of sexual mating. For one thing, it occurred between F^- cells. Moreover, two observations showed that unlike the transmission of traits by sexual mating the transfer did not involve the chromosome itself. First, we noted that known chromosomal traits of certain strains, such as the inability to synthesize particular substances, were not usually transferred along with the drug-resistance traits. Second, we noted that the recipient cells became resistant immediately after the transfer occurred, whereas chromosomal drug resistance is ordinarily expressed only after the original drug-sensitivity genes have been lost in the course of cell division through the process known as segregation.

We concluded that the factor responsible for infectious drug resistance was an extrachromosomal element, which we called the *R* factor (for "resistance"). A number of experiments have confirmed the cytoplasmic nature of these factors. They are obtained by bacteria only by infection from other *R*-factor-carrying cells, never by spontaneous mutation. They can be eliminated from cells by treatment with acridine dyes; *F* factors can be eliminated in the same way when they are in the cytoplasm of F^+ cells but not when they are incorporated into the



INFECTIOUS DRUG RESISTANCE, another form of conjugation, involves transfer of the *R* (resistance) factor. A cell of a re-



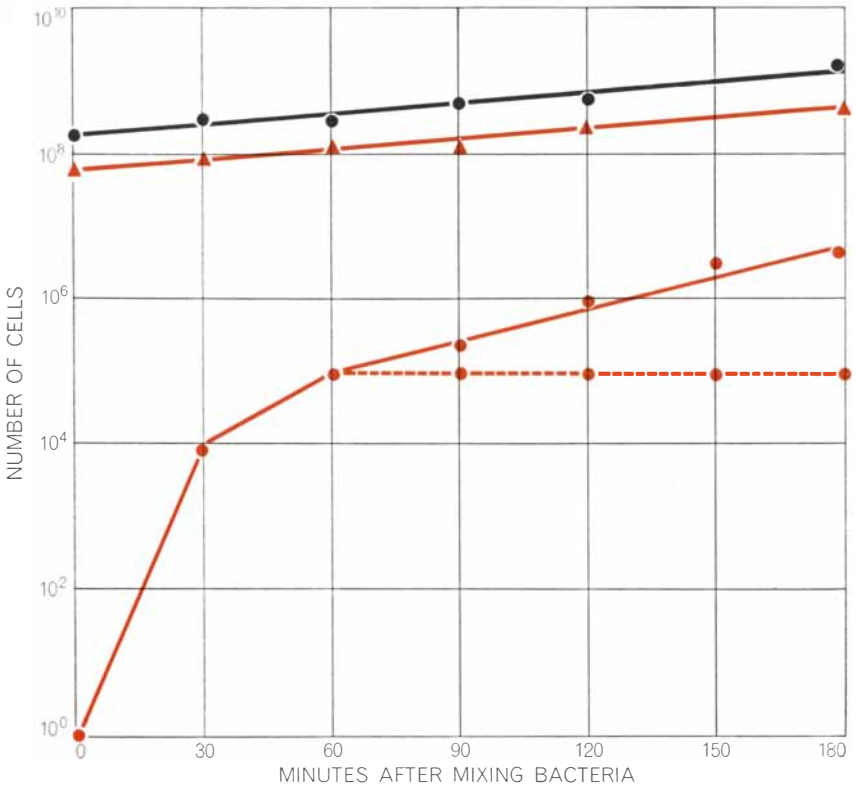
sistant strain (*light color*) comes in contact with one of a sensitive strain (*white*); one of its *R* factors (*color*) replicates and a copy passes through a pilus to the sensitive recipient. The procedure is repeated as cells come in contact. In the course of cell division an *R* factor is sometimes lost. The diagram is highly schematic; the actual sequence of replication and transfer is not established.

chromosome of *Hfr* cells. Finally, consider what happens when one adds a small number of bacteria with *R* factor to a culture of drug-sensitive cells. There is a rapid increase in the relative number of drug-resistant cells; in 24 hours or so the culture is almost completely resistant. This must be owing to the rapid infectious spread of *R* factors to the once sensitive bacteria, because it occurs at a much faster rate than the overall growth of the culture [*see top illustration on next page*]. Since chromosome replication is synchronized with cell division, the *R* factor must be replicating faster than the chromosomes and must therefore replicate outside the chromosome, in the cytoplasm.

Although the *R* factor is usually located in the cytoplasm, in rare instances it is integrated into the chromosome, and when that happens it is transferred together with some chromosomal genes. Such behavior suggests that the *R* factor, like the *F* factor, is episomal in nature. Both of them may be of selective advantage to the cells in which they exist, the *F* factor by making for genetic variability and the *R* factor of course by providing drug resistance. When they are not providing an advantage, they at least do the host cells no harm; they are symbionts rather than harmful parasites. Their behavior is similar to that of a temperate, or nonvirulent, phage, and it may be that both are descended from bacterial viruses. Unlike viruses, they cannot exist at all outside the cell; they are obligatory intracellular symbionts with even less biological function than viruses, which are usually considered to be on the borderline between living and nonliving matter.

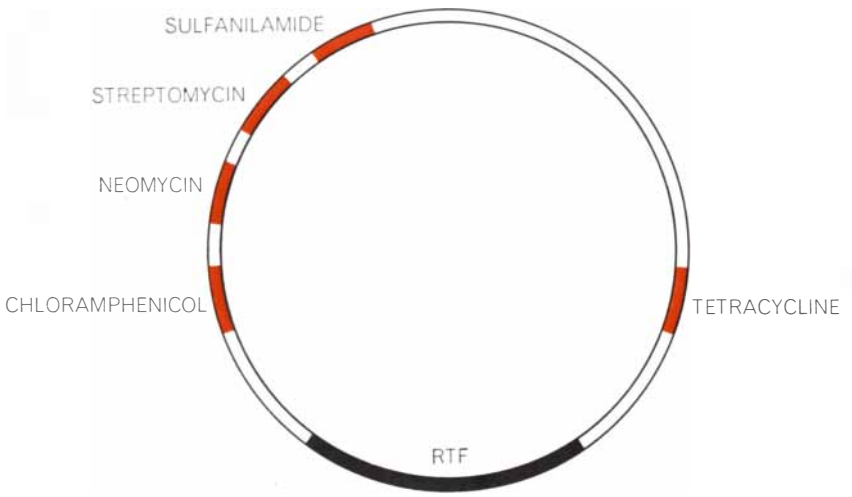
There is a further major point of similarity between the *F* and the *R* factor, and that is their method of transfer. In London, Naomi Datta of the Royal Postgraduate Medical School, A. M. Lawn of the Lister Institute of Preventive Medicine and Elinor Meynell of the Medical Research Council observed in 1965 that most *R* factors induce the formation of pili that are shaped like *F* pili and attract the same phages as *F* pili: apparently they *are F* pili [*see bottom illustration on page 25*]. When bacteria that have such pili and are able to transmit multiple resistance are severely agitated in a blender, the pili are sheared off. Such "shaved" cells are unable to transfer the *R* factor; later, when the *F* pili have been regenerated, the cells are once again infectious. It now appears that both *R* factors and any chromosomal genes mobilized by *R* factors are transferred by the *F* pili or another closely related kind of pili.

The big difference between the transfer of *F* factors and the transfer of *R* factors is in the frequency with which they occur. In a mixed culture of male



TIME COURSE of *R*-factor transfer is shown. Equal volumes of cells of a donor *E. coli* strain infected with *R* factors (*triangles*) and of an initially sensitive recipient strain (*black dots*) were mixed. Sampling at intervals traced the increase in the number of resistant *E. coli* (*colored dots*). After one hour some of the culture was removed, agitated to break off cell-to-cell contact and diluted to prevent pairing. In the diluted culture there was no increase in the number of resistant cells (*broken line*), indicating that conjugation was the mechanism of transfer. The data are from David H. Smith of the Harvard Medical School.

and female bacteria the transfer of nearly 100 percent of the *F* factors or *F*-mobilized chromosome, as the case may be, occurs within an hour. In a culture of drug-resistant (donor) and drug-sensitive (recipient) bacteria, on the other hand, only 1 percent or less of the donor cells transfer their *R* factors in an hour. The low frequency of transfer is due to the relative scarcity of cells with *F* pili in a culture of bacteria carrying *R* factors. Bacteria that have newly acquired the *R* factor, on the other hand, can transfer it at a very high frequency—almost 100 percent. (If this were not the case, *R* factors could hardly multiply so rapidly in a newly infected culture.) They lose this high competence after several cell-division cycles. The explanation seems to be that most *R* factors form a "repressor" substance that somehow inhibits the formation of *F* pili. Cells that are newly infected with such *R* factors contain no repressor, and so *F* pili are initially induced at a high frequency. Later, as the repressor accumulates, the formation of the pili is inhibited.



MAP OF *R* FACTOR, still tentative, shows a closed loop. There are five determinants (*color*) of resistance to five different drugs. There is also a determinant, the resistance-transfer factor (*black*), that controls the ability of the *R* factor to replicate and be transferred.

It is now possible to describe what happens when bacteria with the *R* factor come into contact with a population of drug-sensitive bacteria [*see illustration on preceding two pages*]. A few *R* factors are transmitted by conjugation from donor cells bearing pili into the cytoplasm of recipient cells, which immediately become resistant. The transfer process is repeated from cell to cell, and the normal process of cell division also contributes to the rapid proliferation of multiple resistance in the recipient population. From time to time an *R* factor is lost. Both the rate of transfer and the rate of loss vary in different strains of bacteria and *R* factors, thus accounting in part for the fact that naturally occurring multiple resistance is much more common in some bacteria that are susceptible to infectious drug resistance than in others.

For several years we have been seeking to map the various elements of an *R* factor as one maps the genes of a chromosome. To do this we capitalize on the fact that although *R* factors are not normally transferred by transduction, it is possible to transduce them under carefully controlled conditions. If we grow large phages in a culture of bacteria with *R* factors, a few of the phages pick up entire *R* factors and are capable of transferring them to recipient cells. If we use small phages, there is room for only part of the *R* factor to be incorporated inside their protein coats and transduced. Some of the transduced particles impart drug resistance but lack the ability to replicate or to be transferred by conjugation; others lack determinants of one or more of the multiple drug resistances. By calculating the frequency with which various segments of the *R* factor are transduced together, we can determine their relative distance from one another and so visualize the structure of the *R* factor we are studying.

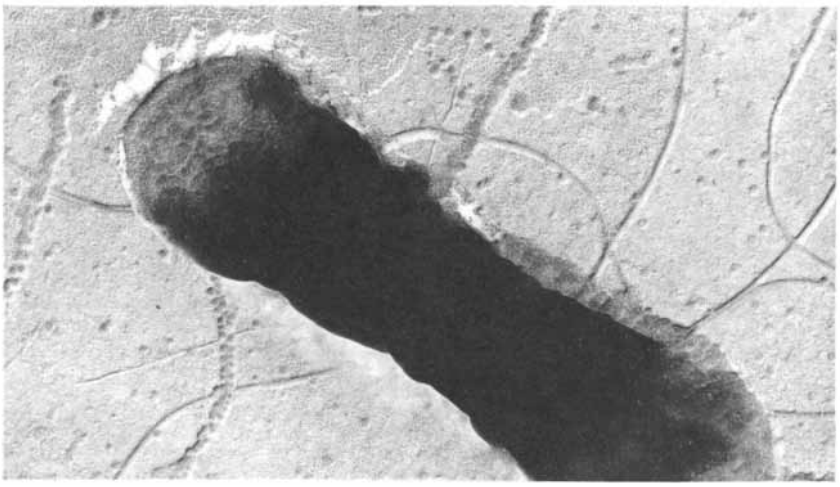
The map is not yet conclusive, but we think the factor is circular and that it has a segment—the resistance-transfer factor, or RTF—that controls replication

and transferability, as well as segments determining resistance to each of five types of drug [*see bottom illustration on opposite page*]. We have suggested that the *R* factors originate when a resistance-transfer factor picks up resistance genes from some bacterial chromosome and that the two then form a single episomal unit. E. S. Anderson and M. J. Lewis of the Central Public Health Laboratory in London have advanced a different view. They consider that the resistance-transfer factor and the set of resistance determinants exist as two separate units, which on occasion become associated to form *R* factors.

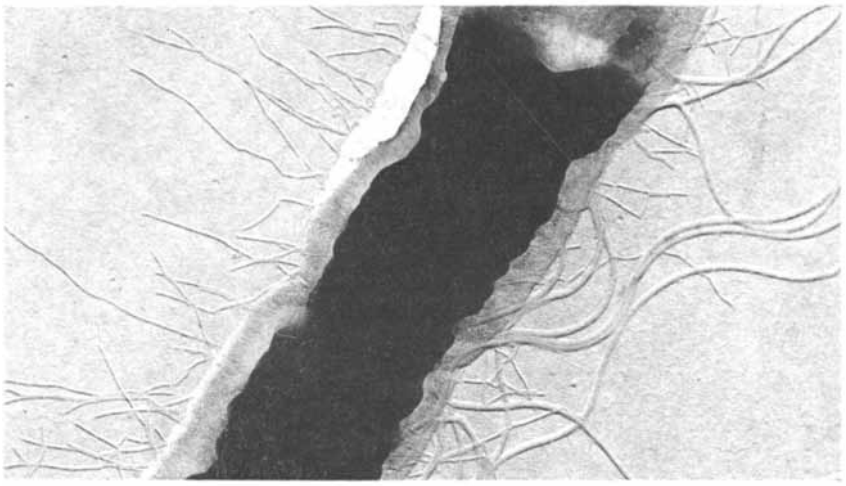
Since *R* factors are self-replicating units, carry genetic information and can recombine with bacterial chromosomes, it is safe to assume that they are composed of DNA. This is confirmed by the fact that *R* factors, like nucleic acids in general, are inactivated by ultraviolet radiation and by the decay of incorporated radioactive phosphorus. At the Walter Reed Army Institute of Research and at Keio, Stanley Falkow, R. V. Citarella, J. A. Wohlhieter and I were able to isolate the DNA of *R* factors by density-gradient centrifugation. A first attempt to separate *R*-factor DNA from that of *E. coli* was unsuccessful, suggesting that the densities of the two DNA's are very similar. We then selected as the host cell the bacterium *Proteus mirabilis*, which was known to have a DNA of unusually low density and to be subject to infectious drug resistance.

When DNA extracted from *Proteus* carrying the *R* factor is centrifuged in a solution of cesium chloride, two satellite bands of DNA appear in addition to the band characteristic of the bacterial DNA [*see illustration on next page*]. These bands disappear if the *Proteus* loses its *R* factors spontaneously or if they are eliminated by the acridine dye treatment, and so we conclude that the bands do represent the *R*-factor DNA. Analysis of this fraction by column chromatography shows that it is typical double-strand DNA. It is possible that *R* factors contain components other than DNA, but this is not likely in view of the fact that entire factors are transduced and transducing phages incorporate only DNA.

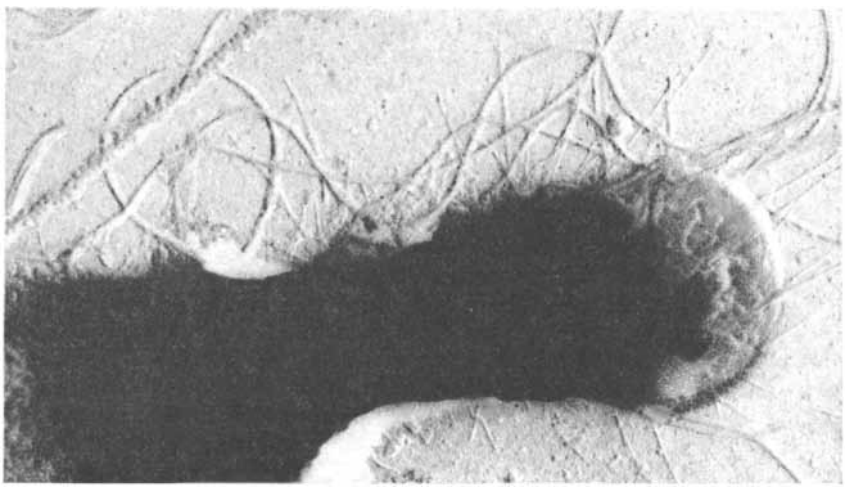
The original finding that infectious drug resistance affected four unrelated drugs implied that some factor was altering the cell membrane, reducing its permeability and thereby barring all the drugs from their normal sites of action inside the cell. The finding that there are separate resistance determinants for the various drugs, however, indicated that



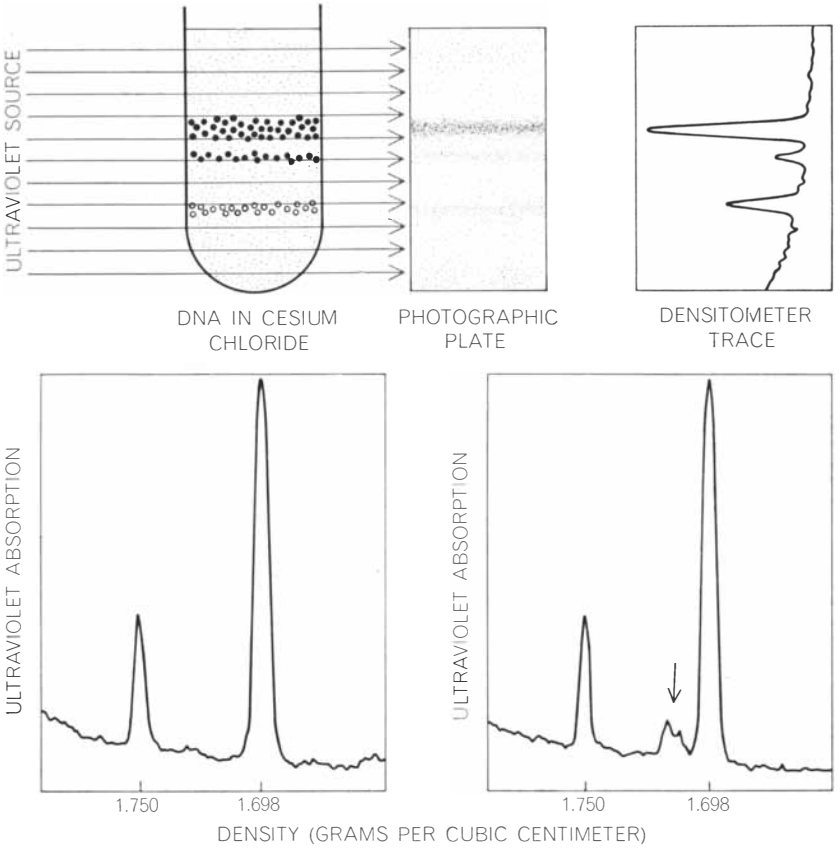
MALE BACTERIUM, an *E. coli* infected with phage, has *F* pili. They are thin fibers, here hidden below the spherical phage particles. The thin fibers without phages are Type 1 pili and the thick fibers are flagella. The preparation has been enlarged about 30,000 diameters.



FEMALE *E. COLI*, which lacks the *F* factor, also lacks *F* pili. It does have both the Type 1 pili and flagella, which are organelles of locomotion for the cell. The electron micrograph, like the others on this page, was made by Toshihiko Arai in the author's laboratory.



***E. COLI* WITH *R* FACTOR, although a female cell, does carry an *F* pilus, the phage-covered fiber at top left. It also has Type 1 pili and flagella. The most common type of *R* factor initially induces the formation of *F* pili, but it also tends to repress them later.**



R-FACTOR DNA is isolated by density-gradient centrifugation. DNA from *Proteus* cells is suspended in a cesium chloride solution and spun in a high-speed centrifuge. The cesium chloride establishes a density gradient and the DNA forms bands in the solution according to its density. The DNA pattern is photographed in ultraviolet, which is absorbed by DNA, and the photograph is scanned by a densitometer (*top*). The densitometer trace derived from *Proteus* without *R* factor (*bottom left*) shows a band at 1.698 grams per cubic centimeter that is characteristic of *Proteus* DNA and a reference band at 1.750. The trace from *Proteus* with *R* factor (*right*) has extra bands (*arrow*) at 1.710 and 1.716, representing *R*-factor DNA.

each determinant had its own mode of action. Permeability may be involved in the case of some drugs, but it is now clear that other processes are at work. S. Okamoto and Y. Suzuki of the National Institute of Health in Japan and Mrs. Datta and P. Kontomichalou in Britain have shown that bacteria bearing various *R* factors synthesize particular enzymes that inactivate specific drugs, thereby rendering them harmless to the bacteria.

The public health threat posed by infectious drug resistance is measured by the range of bacterial hosts it affects, the number of drugs to which it imparts resistance and the prevalence of certain practices in medicine, agriculture and food processing that tend to favor its spread. *R* factors can be transferred not only to shigellae but also to *Salmonella,* one species of which causes typhoid fever; to *Vibrio cholerae,* the agent of cholera; to the plague bacillus *Pasteurella pestis* and to *Pseudomonas aeruginosa,* which causes chronic purulent infections. In addition, more than 90 percent of the agents of urinary tract infections, including *E. coli, Klebsiella, Citrobacter* and *Proteus,* now carry *R* factors.

(These organisms are all gram-negative bacteria; *R* factors seem not to be transferable to the gram-positive bacteria, which include streptococci and staphylococci. A somewhat similar form of transmissible resistance has been discovered in staphylococci, however. There are cytoplasmic genes, or plasmids, in some staphylococci that determine the production of penicillinase, an enzyme that inactivates penicillin. Richard P. Novick of the Public Health Research Institute in New York and Stephen I. Morse of Rockefeller University recently showed that these plasmids can be transduced to drug-sensitive staphylococci both in the test tube and in laboratory animals. The actual clinical importance of this process remains to be determined.)

The *R* factors seem to be acquiring resistance genes for an increasing number of antibiotics. The original factors, it will be remembered, imparted resistance to sulfanilamide, streptomycin, chloramphenicol and tetracycline. In 1963 G. Lebek of West Germany discovered a factor that causes resistance to these four drugs and also to the neomycin-kanamycin group of antibiotics. In 1965 Mrs. Datta and Kontomichalou reported a new determinant of resistance to aminobenzyl penicillin (ampicillin). In 1966 H. W. Smith and Sheila Halls of the Animal Health Trust in Britain found factors imparting resistance to the synthetic antibacterial drug furazolidone. This year David H. Smith of the Harvard Medical School reported *R*-factor-controlled resistance to gentamycin and spectinomycin. We must assume that additional drug-resistance determinants will appear and proliferate as new antibiotics come into use.

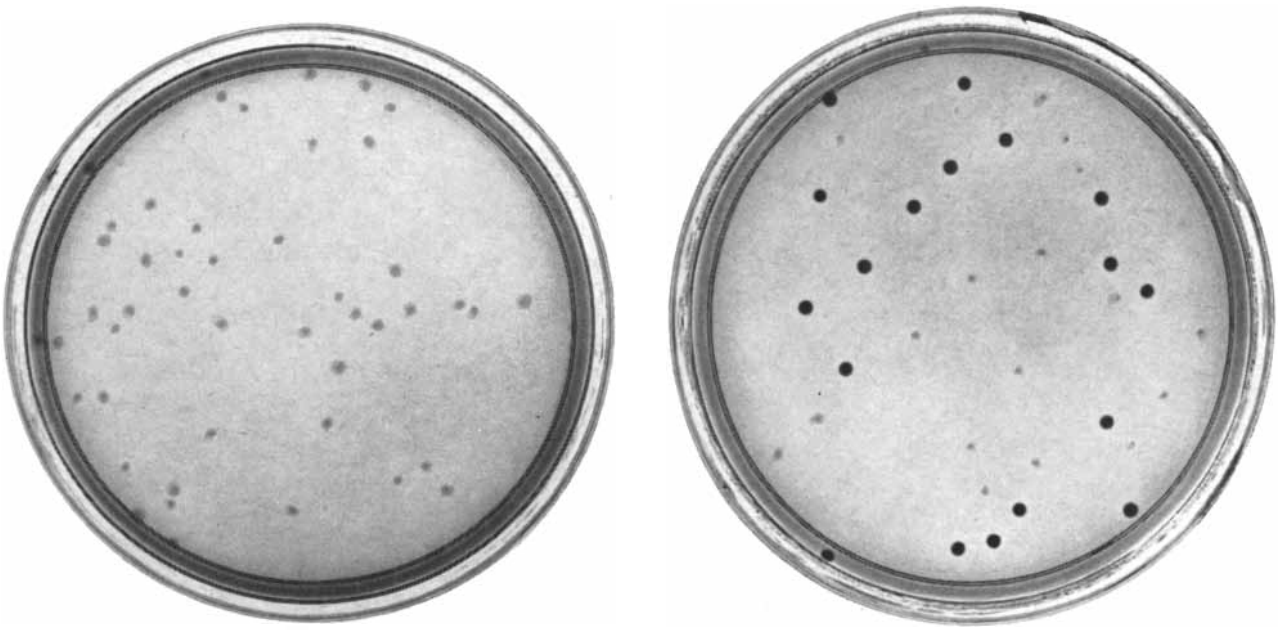
This is implicit in the mechanism of infectious resistance. *R* factors are common in *E. coli,* which are often present in the intestinal tracts of human beings and animals. When a person or an animal becomes infected with a susceptible disease organism, the *R* factor is readily transferred to the new population. Although the frequency of transfer of *R* factors is not high even in the laboratory, and is reduced by the presence of bile salts and fatty acids in the intestine, recipient bacteria bearing the *R* factor are given a selective advantage as soon as drug therapy begins, and they soon predominate.

In addition to being ineffective and helping to spread resistance, "shotgun" treatment of an infection with drugs to which it is resistant causes undesirable side effects. It is therefore important to culture the causative agent, determine its drug-resistance pattern and institute treatment with a drug to which it is not resistant; that is the only way to combat the multiple-resistance strains. As more is learned about the *R* factor, new forms of therapy may be developed—possibly utilizing the acridine dyes, which attack drug-resistant as well as sensitive cells and can also eliminate *R* factors from cells.

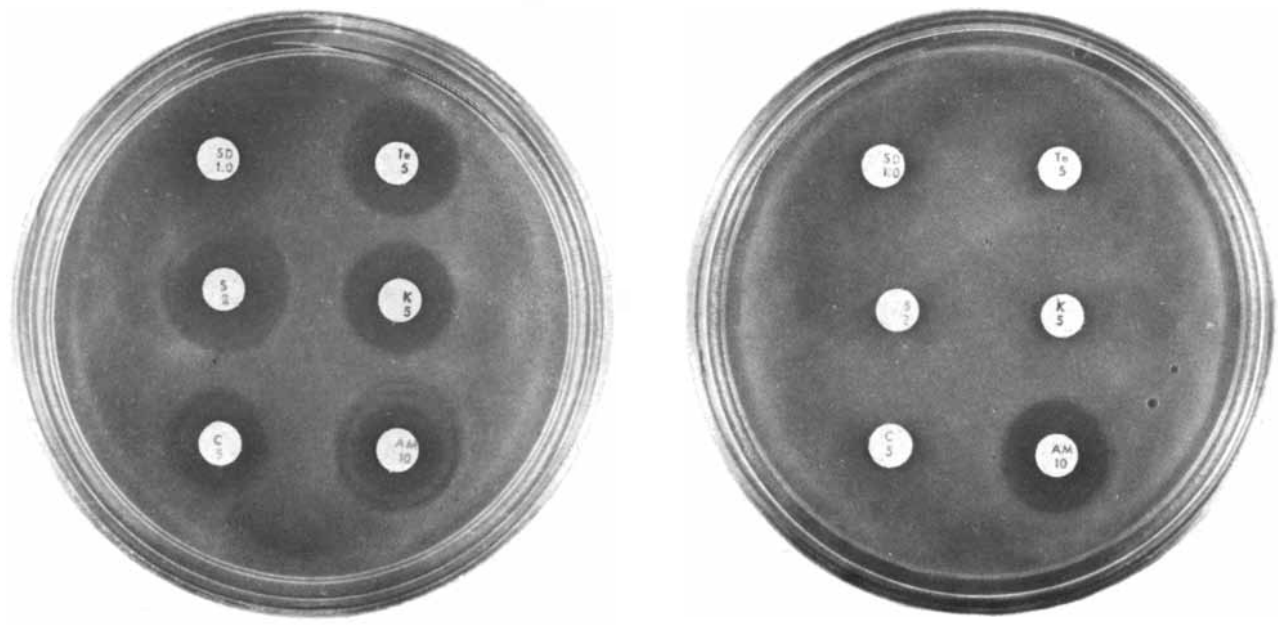
In many parts of the world antibiotics are routinely incorporated in livestock feeds to promote fattening and are also used to control animal diseases. Anderson and Mrs. Datta have shown clearly that the presence of antibiotics in live-

stock exerts a strong selective pressure in favor of organisms—particularly salmonellae—with *R* factors and plays an important role in the spread of infectious resistance. Meat and other foodstuffs are also treated with antibiotics and synthetic drugs as preservatives in many countries, and this too may help to spread *R* factors and carry them to man. Unless we put a halt to the prodigal use of antibiotics and synthetic drugs we may soon be forced back into the preantibiotic era of medicine.

One final note. Typhoid, cholera and plague bacilli are obviously much more difficult to combat if they are resistant to drug therapy. There are grounds for believing that the military in some countries are investigating the potentialities of *R* factors as weapons of bacteriological warfare.



SENSITIVITY TEST conducted in Smith's laboratory at Harvard demonstrates infectious drug resistance. A culture of *Salmonella typhimurium* with an *R* factor controlling resistance to four drugs is mixed with drug-sensitive *E. coli*. A portion of the mixed culture is immediately plated on a medium containing the drugs (*left*). Only *Salmonella* colonies (*gray*) appear. After the mixed culture has incubated, the plating procedure is repeated, and now *E. coli* colonies (*black*) grow as well (*right*): the *R* factor was transferred.



SIMILAR TEST is performed with filter-paper disks impregnated with six drugs: sulfadiazine (*SD*), tetracycline (*Te*), streptomycin (*S*), kanamycin (*K*), chloramphenicol (*C*) and ampicillin (*AM*). A culture of *E. coli* was at first sensitive to all six, as shown by the dark zones around each disk where the bacteria have been killed (*left*). After the culture was incubated with a strain of *Klebsiella*, taken from a patient, that was resistant to all the drugs but ampicillin, the *E. coli* too were resistant to all but ampicillin (*right*).