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EDITORIAL

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Basic research and the challenges of microbiology for the 21st century

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Resistance to antibiotics has become a major concern for microbiologists, biochemists, and physicians worldwide. A report with the conclusions of a Workshop held at Rockefeller University in 1994 showed that several bacterial strains isolated from clinical specimens were resistant to all currently available antibiotics. This set off an alarm as to the dimensions of a possible environmental disaster if there were a diffusion of such human pathogens. These literally drug-immortal bacteria are a serious threat to human populations at the threshold of the 21st century. In 1996 WHO reported that the previous year 33% of the 52 million worldwide deaths were caused by infectious diseases. Since 1973, more than 30 emerging microorganisms (new infectious agents) have been identified. Examples of such new pathogens are: *Legionella pneumophila*, causing legionellosis; *Cryptosporidium parvum*, causing acute and chronic diarrhea; *Vibrio cholerae* O139, a new strain causing the recent cholera epidemic outbreaks; the human immunodeficiency virus (HIV); human herpes virus 8; *Borrelia burgdorferi*, causing Lyme disease; and the Ebola virus. Strong ecological pressures on our overpopulated planet and the free mobility of entire human populations have contributed to the emergence of new infectious diseases.

The "resurrection" of bacterial pathogens is another major concern we have witnessed recently. Among reemerging bacteria are the causing agents of pneumonia (a group of bacteria that cause acute respiratory infections and are responsible for 4.4 million deaths per year), of tuberculosis (more than 3 million deaths per year), cholera, and yellow fever. In the United States, enterococci have become the third major nosocomial pathogen to produce endocarditis. By 1993, vancomycin, which had been an antibiotic used as a last resort against ultra-resistant bacterial strains, was already ineffective against many *Enterococcus faecalis* strains. Similarly, *Staphylococcus aureus* strains resistant to methicillin rose from 8% in 1986 to 40% in 1992.

Reemerging pathogens such as *Neisseria meningitidis* and *Streptococcus pneumoniae* have acquired new strategies for antibiotic resistance based on genetic exchange between different serotypes of certain virulence factors present in microorganisms. Such strategies comprise the formation of mosaic structures which alter the PBPs (penicillin-binding proteins) of the membrane or make it possible for the pathogen to escape the immunological protection acquired by vaccination. Characterization of the genes coding for the formation of the polysaccharide capsules that surround *N. meningitidis* showed that they had been the cause of very virulent outbreaks of meningococcal meningitis detected in the states of Oregon and Washington in the late 1990s. A change from serotype b to serotype c had taken place. The people, who had been inoculated against serotype b, were defenseless against serotype c, and a major outbreak occurred. Epidemic outbreaks caused by *S. pneumoniae*, that originated from similar variations in the capsular types, had also been documented. The complexity of capsulated bacteria adds to the presence of antibiotic resistant genes providing the bacteria with more weapons to develop epidemic outbreaks.

Therefore, both ecological factors and the genetic characteristics of each pathogen play decisive roles in the emergence of infectious diseases. When penicillin started to be widely used in the mid-1940s, pneumococci were very sensitive to this antibiotic. It took some twenty years for the first resistant mutant to be isolated (1967, in Papua-New Guinea). The stabilization of genetic determinants of resistance is usually a long process, but once resistance is acquired, it spreads quickly. In the long run, strategies to defeat reemerging pathogens will have to involve a rational use of antimicrobial drugs as well as changes in social behavior, new public health regulations and information campaigns. Besides, in-depth knowledge of the specific mechanisms of resistance is essential to develop new antimicrobial agents. To acquire this knowledge, basic molecular and physiological research must look into the changes that infectious agents develop continuously in their natural habitats.

The main step that must be taken to fight reemerging diseases is to develop basic microbiology. We must also

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intensify health measures, train health personnel, and promote the search for new antimicrobial drugs, thus expanding our knowledge of microbial diversity. Research on microorganisms that can grow at high temperatures has led to the isolation of enzymes such as the *Taq* polymerase, which have revolutionized traditional laboratory techniques through the extensive use of the polymerase chain reaction. This close relationship with laboratory techniques has demonstrated it is mandatory to prepare scientists that are experts in physiology and taxonomy.

Combining molecular biology and phylogeny is a fundamental step for a better knowledge of the natural microbial world and microbial diversity without the need to isolate and grow species. This kind of research might help us to differentiate between the 3,000 bacterial species described in the Bergey's Manual and the hundreds of thousands of species suggested by microbial ecologists that study microorganisms in their natural habitats. By using molecular probes containing genes that code for rRNA (16S in prokaryotes and 18S in eukaryotes), a new classification of living beings has been postulated. This new classification comprises three large Domains: Bacteria, Archaea, and Eukarya. Homologies among microorganisms established with this new methodology and those coming from the studies in axenic cultures have generated an enormous amount of valuable information. Ecologists agree that this method of analysis of gene sequences will lead to a means of global comparison of microbial diversity, and will facilitate the discovery of new organisms.

Bacteriophages were a fundamental tool in the development of molecular biology and applied microbiology over several decades. Afterwards, interest in studying their economic impact in the food industry seemed to have declined. In the 1990s, however, phages have been again in the spotlight due to the role they play in the transference of virulence genes. This kind of transference and the horizontal gene transfer play a major role in the transmission of "pathogenicity islands," which seem to perform a basic function in the infectious process. The transfer of two genes coding for specific toxins by means of a temperate phage accounts for the virulence of *Escherichia coli* O157:H7, which caused outbreaks of hemorrhagic colitis in the United States, the United Kingdom and Japan. The incorporation of a filamentous phage (CTXϕ) coding for a new toxin to the El Tor pathovar of *Vibrio cholerae* has produced a new strain (O139) still more virulent. The above examples of molecular changes in bacteria are mechanisms that increase both their pathogenic capability and their chances of overall survival. Besides, our chances of exterminating them with the means currently available (vaccines, antibiotics) are continuously decreasing.

A new field of research suggests that bacteriophages might be useful therapeutic agents. This is a revival of the original ideas of the phages' discoverers, at the beginning of the 20th century. Joshua Lederberg has pointed out that this kind of experimental approach is still far beyond our reach due to several limitations in the present technology. He has spelled out, however, the existing limitations in the interactions of microorganisms and mammal cell lines.

Once again, using *E. coli* as a model, scientists have reported that its functions within the intestinal tract can be modified to transform the beneficial microorganism either into an enteropathogen or a uropathogen. Bacterial tropism is determined by a cassette of genes (pathogenicity islands) that is incorporated in a specific point of the chromosome in *E. coli*. Genetic transmission of genes in block has been termed evolution by quantum leaps. Another example of this type of gene transfer mechanism can be found in *Helicobacter pylori*. Strains isolated from patients with gastric ulcer contain a 40 kb fragment of DNA that is absent in the strains obtained from asymptomatic infected patients. Besides, now we know that these pathogenic genes are only expressed when environmental and regulatory conditions are favorable. Therefore, although the virulence genes might be incorporated from an outside organism, the mechanisms that regulate their activity and expression already exist and continue to be functionally active in the recipient cell.

The alarming reemergence of several infectious diseases makes it necessary to establish strong interdisciplinary links between researchers. The study of infection as an ecological process will surely be crucial for the control of microorganisms. Long-term measures should be based both on accurate basic research of the evolution of resistance and on molecular analyses that provide information on the intra- and interspecific transmission of virulence genes. In the meantime, prevention is the most sensible measure—both from the economic and medical point of view—to control infectious diseases.

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