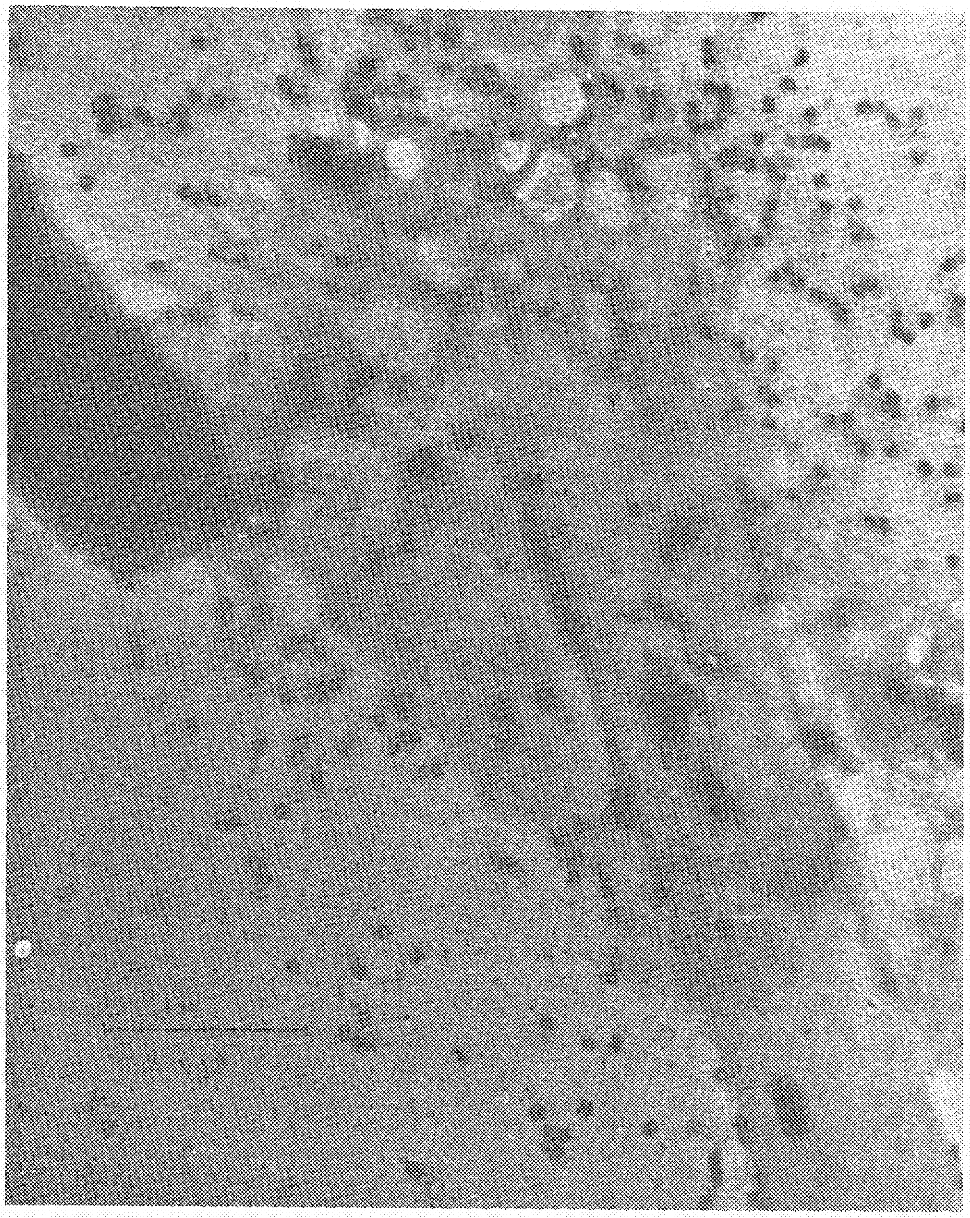


Bacteriophage (black dots), escaping from the corpse of an E. coli bacterium that they have destroyed, ready to attack the next E. coli (upper left). This scene was captured with an electron microscope.

Caltech biologists are using the viruses that attack bacteria to learn more about heredity, reproduction, and the chemistry of life.

by C. M. STEARNS

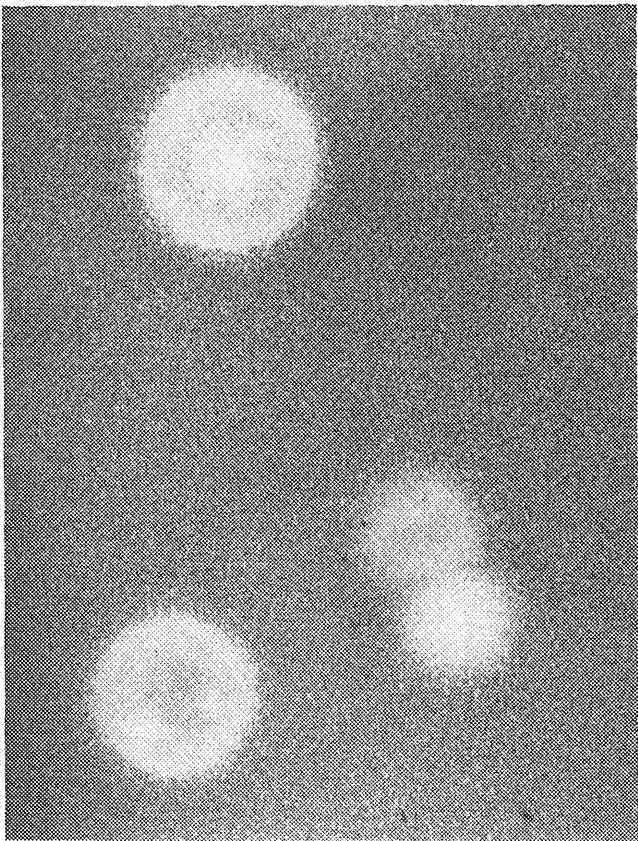
Bacteriophage: A New Test Animal

FROM THE TIME of its discovery during the First World War to the beginning of the Second, the group of viruses called bacteriophage remained little more than a scientific curiosity. In the last few years, however, men like Luria, of Indiana University, and Delbrück and Dulbecco, of Caltech, have lifted the "phage" out of obscurity. It has now reached a prominent position, in laboratories devoted to the biological sciences, as a remarkably useful "test animal" for the study of reproduction, heredity, and the chemistry of life itself.

The virus seems a promising crack in the wall of mystery that surrounds the fundamental process of reproduction principally because it is the simplest system in which reproduction takes place. Small enough to pass through a filter of solid porcelain, without any metabolic system of its own, at times a seemingly lifeless crystalline material, the virus still, once it gets into a living cell, can bring forth copies of itself. In fact, the virus is little more than a pure self-reproducing principle.

But even so, virus reproduction is not easy to study. For one thing, a virus will not reproduce outside of a living cell. The study of its reproductive mechanism is therefore obscured by the chemistry of the cells within which the virus multiplies, and frequently by the chemistry of the whole organism (for instance, a man) of which those cells are a part.

This difficulty was partly overcome when, a number of years ago, biologists began working with a group of viruses that attack, not the cells of complex animals, but the relatively simple single-celled bacteria. This group of viruses has been given the collective name of bacteriophage.



Four holes made in bacterial colony by phage. Size and clearness of "plaques" identify phage strain at work.

Because the bacteriophage virus attacks bacteria, its discovery raised great hopes that it might offer a cure for bacteria-caused diseases. It would be a simple matter, doctors thought, to dose a patient with bacteriophage and let the "phage" kill the guilty bacteria. For a number of reasons, the scheme failed. But bacteriophage, because it operates at such an elementary level of life, has made up for its early failure by becoming an ideal tool for studies of reproduction, in laboratories at the Institute and elsewhere.

For the sake of obtaining uniform results, one group of scientists who work with bacteriophage have limited themselves to seven closely related strains, all of which attack one kind of bacterium. The bacterium involved is Escherichia coli (bacteriologists habitually shorten the first word to "E"). E. coli's natural habitat is the intestinal tract of man and other animals; it is almost always there and almost always harmless. But E. coli thrives also in the test tube, or on the rimmed glass discs known as Petri dishes.

In ordinary studies of the phage's reproductive processes, the phage's attack on E. coli is not observed directly. It is possible, however, to watch the process. Under a microscope with "dark field illumination," E. coli look like miniature capsules, and the phage particles are no more than minute flashes of light a little like minnows in a sunlit pond. Focus on one individual E. coli bacterium, and after a few minutes you will notice that it seems to be boiling, inside. It is not possible to see the phage entering the bacterium (how it enters is, in fact, one of the still-unsolved problems of phage research); but the boiling is proof that a phage has entered the bacterial cell and begun to reproduce.

Then, suddenly, after perhaps twenty minutes, the E. coli cell bursts wide open, and a watchful eye may see a whole cloud of new phage particles moving away from the broken corpse—each new phage ready to find another E. coli and repeat the process. One phage, hidden inside a bacterial cell for fifteen or twenty minutes, has somehow multiplied itself to 300 or more.

But, as in most similar studies in current biological research, bacteriophage experiments rely not so much on individual viruses attacking individual bacteria as on the statistical results of thousands of attacks. For example: to discover the effect of some chemical agent on the reproductive power of a phage, it is not necessary (and would in fact be impossible) to inject the agent into one phage and then persuade that phage to enter a single bacterium. Instead, the agent can be mixed with fluid containing thousands or millions of phage, and the mixture applied to E. coli growing on Petri dishes. Thereafter, a simple count of the clear spots, or plaques, on the Petri dishes gives the answer. Each plaque represents an area where E. coli has succumbed to phage, and the number of plaques is therefore a key to what effect the agent being tested had on the phage's ability to multiply. The whole test procedure is characterized by biologists, respectfully, as an "elegant one," meaning that they admire its ingenuity, its simplicity, and the lack of ambiguity in its results.

The Chemical Approach

Another way of approaching the problem of reproduction as it occurs in bacteriophage is the chemical way. Chemical analysis of the nature of phage shows that it is made up largely of phosphorus-containing proteins. So chemists have added radioactive ("tagged") phosphorus to a medium containing E. coli infected with phage, and at suitable intervals have measured (with Geiger counters) the relative amounts of "hot" phosphorus that remain in the medium, that have been taken up by the bacteria, and that have been taken up by phage. By this technique chemists have begun to separate the steps that the phage takes, during reproduction, to build up the phosphorus-containing proteins needed to make up the new phage particles.

The experiment has recently revealed that what a phage actually does is to take over the metabolic system of the E. coli it has penetrated and put it to work making, instead of more E. coli material, more phage material. And this, by the way, is getting quite close to the kind of thing that may go on when the gene of the human cell uses the material surrounding it to make a second gene like itself to be transmitted to the cell's offspring. It is getting even closer, of course, to what must happen when a virus, say that of polio, penetrates the nerve cell of a victim.

Inseparably bound with the problems of reproduction are those of heredity, for heredity is in the final analysis simply something that results from the process of reproduction. And in understanding heredity, too, the bacteriophage is therefore useful. The seven strains of phage that scientists use invariably breed true to form in certain ways—in shape, for example, and in the length of time that it takes for a phage to multiply and burst the bacterium that it has invaded. For any given one of the seven strains of bacteriophage, these particular traits remain unchanged through generation after generation.

But there are a few traits that change. One controls the size of plaque that each strain of phage can produce in a Petri dish of E. coli; a strain that normally (in the "wild" state, a biologist would say) limits itself to small plaques may begin to make larger ones. Another change-

CALTECH BACTERIOPHAGE GROUP, 1950

Gunther Stent H. M. Kalckar Max Delbruck Leora Duberg
Egon Bittner G. H. Bowen William Hudson Renato Dulbecco
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able trait is defined by the range of E. coli types that a strain of phage attacks; a phage strain may suddenly increase in its ability to overwhelm differing kinds of bacterium. The fact that there are a few, but only a very few, ways in which bacteriophage are known to mutate makes them seem as promising to the investigation of the fundamental machinery of heredity and mutation as, say, the fruit fly, which is the old and valued stand-by of geneticists—but which exhibits instead of a few, more than five hundred genetic variations.

There is one further way in which bacteriophage may prove useful in the study of reproduction and heredity, and in fact in the study of a process so basic that it applies to several other aspects of cell behavior as well. This process is the one by which substances outside a cell manage to pass through the cell's membrane and get inside, and is obviously a fundamental part of the chemistry of life.

In the case of the phage, the problem centers around the question of how a phage gets into a bacterium. The reason why it may be possible to answer this question is this: while most phage have no trouble getting through E. coli's protective sheath, there is an occasional mutant form of phage that requires outside help. This helpless variety cannot break into E. coli unless there are added to the medium surrounding E. coli certain specific chemicals, such as tryptophane. If it is possible to discover why tryptophane is able to assist a phage to pass the bacterial wall, the answer may throw helpful light on the whole problem of cell penetration.

Such is the scientific position of the bacteriophage, and such is its promise. What has it proved, so far?

First of all, the phage has proved that something quite like sexual reproduction goes on in the interior of an E. coli that it has attacked. It was once supposed that, at this simple level of life, reproduction followed the familiar nonsexual system of division—one unit dividing to give two, the two dividing to give four, and so on. In such a pattern of reproduction, each cell (barring mutation) has of necessity only those characteristics that were possessed by its one "parent."

In the sexual pattern of reproduction, however, *two* parents contribute traits to the offspring. And it has been found that when E. coli is infected with two types of phage, the cloud of new phage particles that later bursts from the destroyed bacterium contains many phage that combine the traits of both of the two parent types. This indicates, then, that there must be at least some similarity between the hereditary machinery of the phage and that of infinitely more complex systems such as the human being.

Another surprising field of investigation has been opened to view through the study of the effect on bacteriophage of ultraviolet light. Ultraviolet light of a certain wavelength can "kill" bacteriophage, so that it is no longer capable of reproducing and destroying E. coli. That is not surprising, since ultraviolet light has the power to damage a wide range of microscopic germs and spores, and even the living cells of the human eye (as many people have found by looking too long at the sun). What *is* surprising is the recent discovery that ultraviolet light of a slightly different wavelength can bring the killed phage back to life.

This discovery has raised many questions and answered none, which is just what a great many important scientific discoveries have done in the past. But it may open a new way to the understanding of what makes a phage active and thereby help to disclose a new link in the chain of events that constitute life.

Practical results from the phage research now in progress at the Institute (and elsewhere) are far, perhaps years, off. But, as the foregoing discussion of the procedure and promise of phage research has attempted to show, the phage may someday assume a scientific importance out of all proportion to its size—which is such that it would take between two and four hundred thousand phage, side by side, to make a line one inch long.