VIRUSES

The very small microorganisms that grow only in cells are studied not only for the treatment and prevention of the infections they cause but also for themselves

by F. M. Burnet

POLIO, influenza and the common cold—probably the three infectious diseases of most interest to the average person—are all caused by viruses. So are smallpox and yellow fever, most of the "childhood diseases" and a host of rarer maladies. Since the days of Jenner and Pasteur the virus plagues have been studied from every angle that might help toward their understanding and control. It is natural that most of the research in this field should have a strongly medical bias. In dealing with disease, as in every important human problem, it is of more immediate value to find some effective answer than to have a clear understanding of the nature of the problem. The history of yellow-fever research provides a good example. Walter Reed's famous experiments in 1900, which proved on the bodies of U. S. Army volunteers that yellow fever was carried by a certain species of mosquito, provided all that was necessary for the control of yellow fever in the West Indies. But the nature of the microbe carried by the mosquito was not discovered until 1928. For a time the culprit was thought to be a bacterium. Then for five or six years, on the authority of the great Japanese-American bacteriologist Hideyo Noguchi, the germ was very widely accepted to be a species of Leptospira, a coil-shaped microorganism in the general class that includes the spirochetes. Finally, as a result of work in West Africa by a Rockefeller Foundation team, it was conclusively proved that yellow fever was due to a virus. Noguchi himself confirmed this finding—and died of yellow fever contracted in the laboratory before his work was completed.

There are similar stories of pragmatic research, with varying failure or success, to be told about all the major virus diseases. But along with this work on prevention and treatment there has been developing in recent years an increasing attention to the fundamental nature of virus infection. There are two excellent justifications for fundamental research. It is the only attack that is likely to open up unexpected new approaches to the practical problems, and it satisfies that almost mystic desire to do something toward seeing the universe "all of one piece." Also, for a variety of reasons which someone might find it interesting to analyze, most scientists worth their salt seem to get more straightforward fun out of basic research than out of anything else.

Quite apart from the problems of human and animal disease, the viruses themselves—their nature, their interaction with the cells they infect, their place in the evolutionary scheme—provide topics of the highest interest. This article is an attempt to give an account of modern experiments and ideas that bear on these matters. It will be based to a considerable extent on the investigations of influenza virus that have gone on in England, America and Australia during the last 17 or 18 years. The influenza viruses are my own chief field of interest, and they are also the field in which fundamental study of animal viruses—as opposed to the viruses that attack plants or bacteria—is furthest advanced.

The Nature of the Beast

A virus can be defined as a microorganism, considerably smaller than most bacteria, which is capable of multiplication only within the living cells of a susceptible host. This definition immediately indicates the important feature that distinguishes the virologist's problem from that of the classical bacteriologist. A bacterium, say the diphtheria bacillus, can be grown on relatively simple mixtures of sterilized nutrients—the tubes of broth and the plates of nutrient agar that are the bacteriologist's tools of trade. For viruses nothing less than the living cell will serve. An influenza virus can be grown in the nasal passages of a ferret, in the lung of a mouse, in the tissues of a developing chick embryo or in a culture of embryonic cells in a flask, but it will not grow in any nonliving material.

There are two general prerequisites for experimental laboratory work with a man-infecting virus. First, the experimenter must find some convenient animal whose cells can be infected by the virus. If chick embryos or mice, which are cheap and available in virtually unlimited number, will serve, so much the better. Second, the experimental host must show some sign or symptom that will allow the experimenter to know when it is infected.

Any good experimental work must be quantitative. In most experiments with viruses the questions we ask usually take the form of an inquiry as to how much virus is present after such and such a manipulation. Suppose we are working with influenza virus in mice and wish to know how much virus is present in an extract from the lung of a mouse that has just died of the disease. Our method of measuring this depends on the amount of consolidation (solidification) of the lungs produced by various doses. If we put a large dose of the virus into the nose of a mouse, it will die in a few days with the entire surface of its lungs consolidated. Smaller doses will produce consolidation of only a portion of its lungs. We can adopt a convention that one unit of virus is the amount which on the average produces consolidation over 50 per cent of the visible surface of the lungs. To measure the strength of our extract from the lungs of the fatally stricken mouse, then, we dilute the extract in varying degrees, so that we have samples diluted to one part in 10, one in 100, and so on. Each of these samples is inoculated into the noses of six mice four to five weeks old. A record is kept of deaths in each group and of the aspect of the lungs when the surviving mice are killed seven days after inoculation. If we find that 50 per cent consolidation

occurs, on the average, in mice given a 1-to-10,000 dilution of the extract, while other doses produce more or less consolidation, the original extract is reckoned to have a strength (titer) of 10,000 units of virus. This principle of diluting something down in a series of steps until it produces a certain standard degree of action is very cumbersome in practice and not very accurate. Furthermore, when the titer has to be measured by its effects on monkeys, as in poliomyelitis research, or on human volunteers, as in the study of colds, the process becomes enormously expensive. But so far no more convenient method of measurement has been found, and for most viruses the dilution technique will probably remain the standard quantitative procedure.

The fact that research on influenza virus is much further advanced than research on poliomyelitis is very largely due to the greater facility of measurement. The action of influenza virus can be measured not only in the mouse and the chick embryo but also in the test tube. When the virus is mixed with a suspension of red blood cells in saline, it causes the cells to clump in easily visible fashion. George K. Hirst, then of the Rockefeller Institute for Medical Research (he is now at the Public Health Research Institute of New York City), discovered this agglutination phenomenon in 1941 through a lucky accident. He was examining chick embryos that had been infected by injection of virus into the fluid-containing cavities that surround the embryo. The fluid in these sacs was known to be very rich in virus. During such an examination some infected fluid, mixed with a little blood, usually spills over into the dish. Hirst noticed that the blood cells in the spilled mixture collected into coarse clumps. It is the mark of a first-class investigator to see the implications of an unexpected occurrence. Hirst immediately grasped the potential importance of his observation. If the virus, or something associated with it, could produce this easily visible effect in the test tube, here was a direct means for recognizing the virus' presence and measuring its amount. It was a relatively simple task to devise an appropriate test along these lines. In the course of this work it was established that the virus itself produced the effect on the red cells. Since then various applications of the agglutination technique have made it possible to analyze the qualities by which one type of virus differs from another.

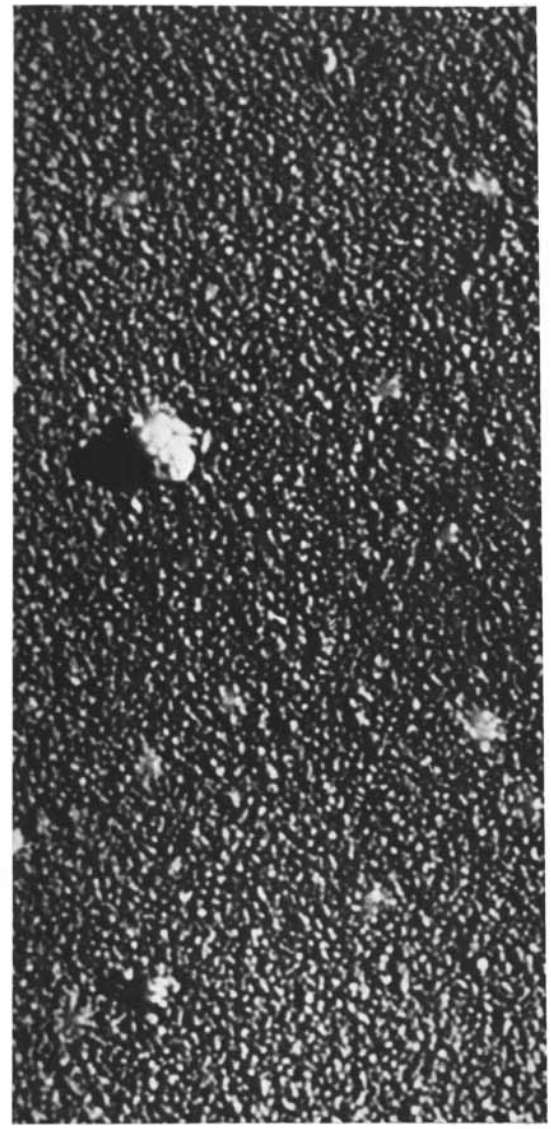
How It Attacks the Cell

The very fact that the influenza virus agglutinates blood cells has provided the most important of all leads to an understanding of how the virus makes effective contact with the cell it is going to infect. Red blood cells themselves are not susceptible to penetration and infection by influenza or any other virus. But the surface of the red cell seems to have essentially the same complex mosaic of chemical components as any other cell from the same species of animal. There is much direct evidence that the action of an influenza virus on the surface of red cells corresponds closely to its action on the susceptible cells that line the air passages of a ferret or a mouse. Experiments with red cells may therefore provide a convenient model of what happens in the more important but less accessible tissues of the lungs and bronchial tubes.

From large numbers of experiments in many laboratories we have a fairly clear picture of the process by which an influenza virus initiates infection of a cell. The virus seems to approach the cell surface through a reaction closely resembling that between an enzyme and the substance it acts upon. The virus particle has on its surface a number of patches which function as enzymes. These enzyme patches attach themselves to and break down certain molecules of a complex carbohydrate that are built into the surface of the cell. The virus can then sink into the substance of the cell and there begin to multiply.

The points on the cell surface to which the virus attaches are spoken of as receptors, and the complex carbohydrate of which they are composed belongs to the class called mucins or mucopolysaccharides. These are sticky substances like those responsible for the stickiness of egg white and saliva or for the slime track of a snail. The receptor mucin is closely related to the mucins that form a protective film over all the moist air and food passages, provide the chemical basis for the blood groups A, B and O and serve as one of the most important of the sex hormones, gonadotrophin. Influenza virus acting as an enzyme, it has been found, will rapidly destroy the activity of the hormone responsible for the sexual development of the immature female rat or mouse—here surely is a most unexpected crossing of paths between two distinct fields of biology.

In the course of work in my laboratory in Melbourne we found that the organism responsible for cholera produces an enzyme of the same type as the influenza virus enzyme. This enzyme is not part of the cholera germ but is set free in soluble form and can be concentrated and purified by chemical methods. We call it RDE (receptor-destroying enzyme). The isolation of this substance provided an opportunity for a very interesting experiment. If RDE destroys the cell receptors, and if influenza virus can enter cells only through such receptors, then an injection of RDE should make an animal immune to influenza. Joyce Stone of our laboratory performed the experiment both on mice and on chick embryos and found that this



INFLUENZA VIRUSES are made visible as small white spheres by an

was indeed the case. The immunity is very short-lasting, however, for the cells regenerate fresh receptors within two or three days.

So far this mechanism of cell entry by viruses has been definitely established only for viruses of the influenza group. But within the last year somewhat similar observations have been made on two groups of viruses closely resembling, but not identical with, the poliomyelitis viruses; mice have been protected against infection by one of these types of virus by prior treatment with RDE. It is too early to say whether developments in this field will have any significant influence on the prevention or treatment of virus diseases of man. There is nothing of the sort immediately in sight, and for the time being research of this kind must look for its justification more in the interest of the problem itself than in the promise of medical or economic benefit from its solution. But the work done so

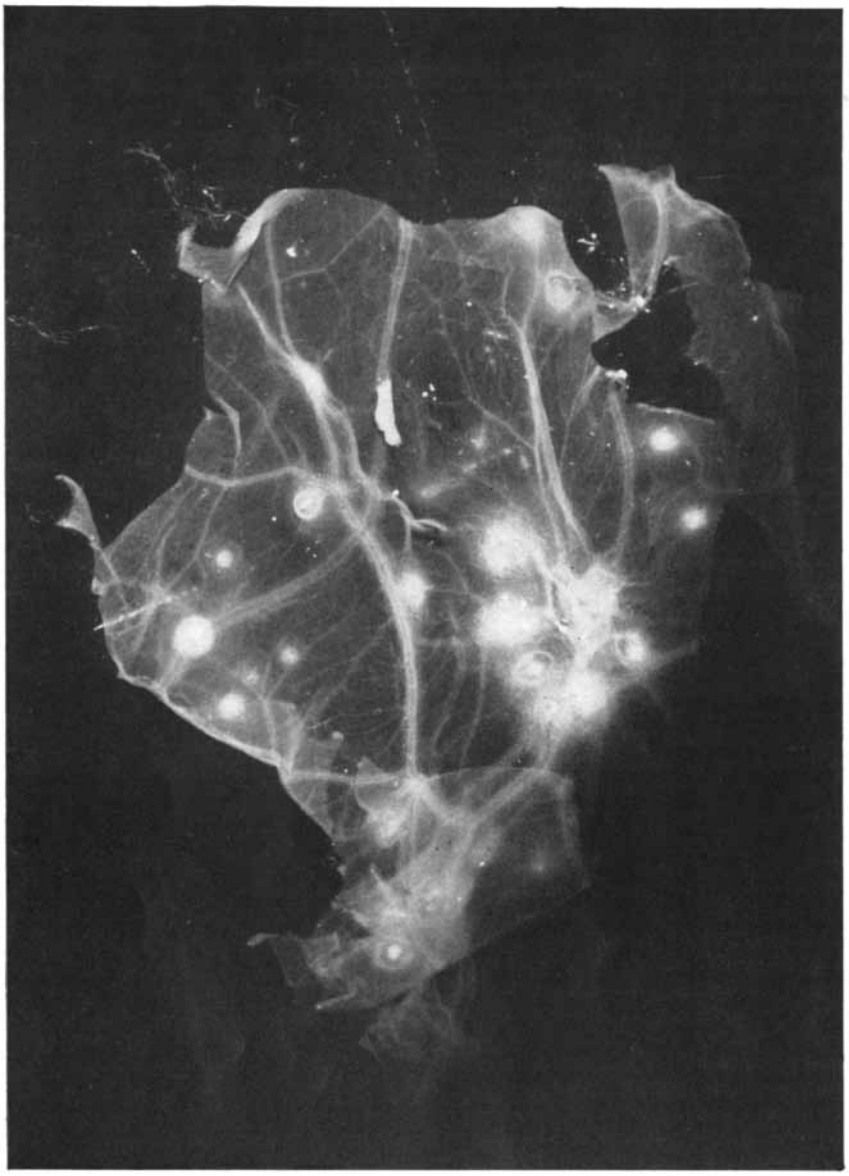
electron micrograph. The rod-shaped particle at top center is a filamentous form of the virus. This micrograph, which enlarges virus particles 47,000 times, was made by R. W. G. Wyckoff of the National Institutes of Health.

far suggests that the chemistry of cell entry by viruses may eventually become of great importance to workers in virus diseases.

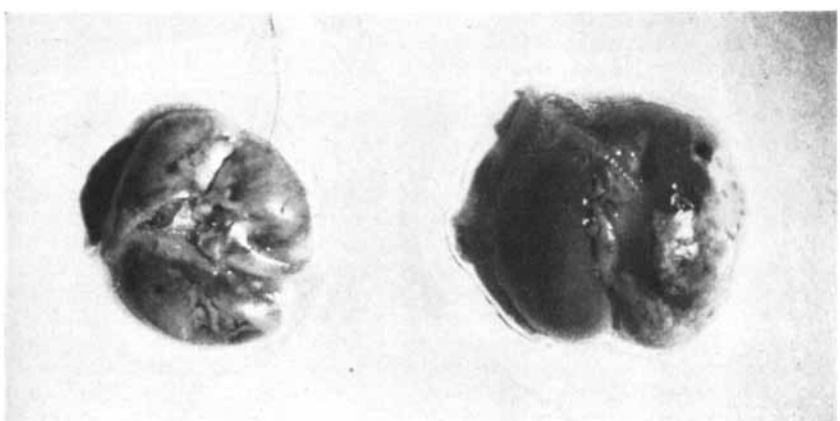
Changes in the Breed

When a virologist undertakes an investigation of a human disease, his first concern is to find some laboratory host for the virus. His next is usually to "hot up" the virus for the new host so that it will regularly produce whatever symptom or lesion is being used as an index for the presence of the virus. Only rarely does fresh virus from a human patient multiply easily in the laboratory animal. Ordinarily it must be adapted to the animal by a series of transfers, or "passages," from one individual to another in the new host. It follows, therefore, that what the virologist works with is strictly speaking not the human virus he started with but a variant—a laboratory-adapted variant. Sometimes the difference may be very striking indeed. The stock influenza viruses of the laboratory are studied mainly by observing their capacity to produce pneumonia in mice or to agglutinate red blood cells in chick embryos. Influenza virus A as it comes from the human throat is quite incapable of doing either of these things. Similarly the virus used for vaccination against yellow fever, though a live, lineal descendant of a fatal virus taken from a patient who died, produces no illness at all, because it has been changed into a harmless strain by passage through chick embryos.

The capacity of viruses to change their character in nature or in the laboratory is obviously of the greatest practical importance. It is accomplished by the processes of mutation and selective survival. The human influenza virus becomes capable of growing freely in the chick embryo not because it has gradually learned how to do so but because random mutations have provided virus variants from which those most capable of surviving and multiplying in the chick embryo have been selected. Laboratory experiments have definitely proved that this is the case, not only for influenza viruses but also for bacteriophages (viruses that attack bacteria). There is much evidence, too, that influenza virus mutations occur in nature and play an important part in determining the timing and extent of epidemics of influenza. For some reason the influenza viruses appear to be especially mutable. This can be positively embarrassing at times. A standard influenza virus is sent to two laboratories which maintain it in slightly different fashions. At the end of 10 or 15 years the descendant viruses in the two laboratories may differ very considerably, and these differences can create confusion or even ill-feeling when investigators, thinking they are working with



COLONIES OF VIRUS grow as small white "plaques" on a membrane of the chick embryo. The virus is vaccinia, the close relative of the smallpox virus that infects cattle and is used to vaccinate humans against smallpox.



MOUSE LUNGS are a means of measuring the amount of influenza virus in a given extract. At left is a normal mouse lung; at right, an infected lung. Darker, or consolidated, areas are a measure of the amount of virus.

the same virus, obtain discrepant results.

Most present-day biologists would agree that the most fundamental aspects of living processes are all related in one way or another to the problems of reproduction and variation—the subject matter of genetics. If we are to get to grips with the real nature of viruses, it will be necessary to have a genetic approach here, too. The necessary beginning is to obtain a wide range of mutants with each variant as pure as possible, *i.e.*, at least 99.9 per cent of its population uniform. This is relatively easy to do in the case of influenza virus, which produces spontaneous mutations so readily that there is no difficulty in obtaining most of the mutants one desires. For other viruses the artificial acceleration of mutation by radiation or chemical treatment may become important, not only as an aid to research but also to produce variants that can be used for immunization against the diseases caused by these viruses.

The Virus in the Cell

Until recently it was a convenience to believe that viruses lived and reproduced very much like small bacteria; that is, that they multiplied by the same process of enlargement and division and differed from bacteria merely in the fact that they required a more complex nutrition, which only the interior of the living cell could provide. Today it seems that this is almost certainly incorrect. The virus actually multiplying in the cell is something quite different from the virus that passes as the infectious agent from cell to cell or from person to person. We do not yet understand the process that takes place inside the cell; when understanding comes, it may throw a flood of light on some of the most important aspects of fundamental biology.

This idea that a virus within the cell is distinct from the infectious particle whose picture is given by the electron microscope came first from studies of the viruses that attack bacteria—the bacterial viruses, or bacteriophages. Like the viruses that cause disease in man or animals, the bacterial viruses are incapable of multiplication except within the cells they infect. The virus particle first makes a chemical union with some component of the bacterium's surface and then by some process penetrates the cell wall and finds itself within the cell substance. After it has made this entry, the virus vanishes for a time; we can find no sign of its presence by any test. What happens can only be judged by indirect evidence. We observe, for instance, that when viruses are damaged beforehand by treatment with ultraviolet light, they can somehow combine their materials in the bacterium to produce whole offspring. Moreover, when two different viruses are made to infect the same bac-

terium, they yield a mixed inheritance, including forms that can only be interpreted as hybrids. From these evidences we conclude that the virus on entering the cell liberates or breaks up into a number of subunits, which are sufficiently analogous to the bearers of genetic characters in higher organisms to be called genes. Each gene multiplies more or less independently until a large "pool" of genes is created at the expense of the bacterial substance. Then from the pool groups of genes begin to aggregate in such a way that each group contains all the genetic components needed for the construction of the virus particle. Once formed, the group becomes a center of organization that draws to itself the material needed to complete the formation of a virus particle of the new generation. As new virus accumulates, the bacterium wastes and weakens until there is a sudden collapse of its structure, with liberation of 100 or more new virus particles.

The fact that this mechanism provides a means of combining the properties of two different viruses is a point of particular interest. The genes contributed to the pool by both parents are re-sorted and may appear in various combinations in the groups of offspring. Actually not only two but three or even more different viruses entering a bacterium may combine some of their hereditary characteristics in a single virus particle among their progeny! Hybridization is hardly an adequate term for such a process.

There are as yet no studies on animal viruses to match this work on the bacteriophages. Influenza virus, however, has been extensively studied along similar lines, and it is extremely likely that the eventual interpretation of its process of multiplication will be almost identical with that of the bacterial viruses. It has been found, for instance, that when an influenza virus is grown in one of the cavities of the chick embryo, the virus particles become attached to the cells within an hour; then they disappear until a fresh generation of descendant particles is liberated from the cells between five and eight hours after infection.

In the case of influenza virus, treatment of the particles with ultraviolet light can interfere with the multiplication of living virus in the embryo. It seems that this "killed" virus—killed at least in the sense that it never multiplies in susceptible cells—can often enter a cell and in some way block a component of the cell that is necessary for the reproduction of active virus. This interference effect also occurs when two living viruses infect a cell, if proper experimental methods are used. It plays an important part in the experiments which we shall now have to discuss. These provide more definite evidence as to how influenza viruses multiply.

In experimental biology one can often learn more about the working of an organism by observing its behavior in some alien environment than by watching it in its normal place in nature. We have obtained our most interesting results by injecting influenza virus into the mouse brain, which is even farther than the chick embryo from the virus' natural habitat—the human air passages.

Viruses in Foreign Cells

When an ordinary influenza virus is injected into a mouse's brain, even in rather large amount, the mouse may show some evidence of sickness for 24 hours but later recovers completely. The virus is not inert; some sort of abortive multiplication must take place, for the amount of virus often increases slightly in the first 10 or 12 hours and it does not disappear entirely until four or five days later. R. Walter Schlesinger of the New York Public Health Research Institute has obtained strong evidence that when virus enters "alien" cells, instead of multiplying in normal fashion it gives rise to something which may be called "partial virus." His finding was that the blood-agglutination test indicated a much larger amount of virus substance to be present in the cells than did the standard chick-embryo and mouse infection tests. This suggests that the virus offspring in the alien cells retain the ability to agglutinate blood but have weakened in their power to infect. The conception of "partial virus" is not easy to grasp, and many virologists are chary of offering any detailed interpretation of Schlesinger's facts. But his finding fits in rather neatly with the results of our mouse-brain experiments.

Although no ordinary influenza virus can infect the mouse brain, about 12 years ago a combination of accident and "training" in a laboratory in England did produce a strain of influenza virus that could multiply freely in a mouse's brain and kill the animal. This strain, which remains an influenza virus in every respect except its unusual ability to infect brain cells, we have named "neuro-flu" virus. It can be grown quite normally in chick-embryo cavities, giving highly infectious fluids for experimental use.

When highly diluted "neuro-flu" virus is inoculated into the brains of a group of mice, the animals appear quite normal as soon as they recover from the anesthetic. But after four or five days they begin to sicken, and a day later they die with signs of brain infection. Tests of the brain show that it contains very large amounts of fully active virus.

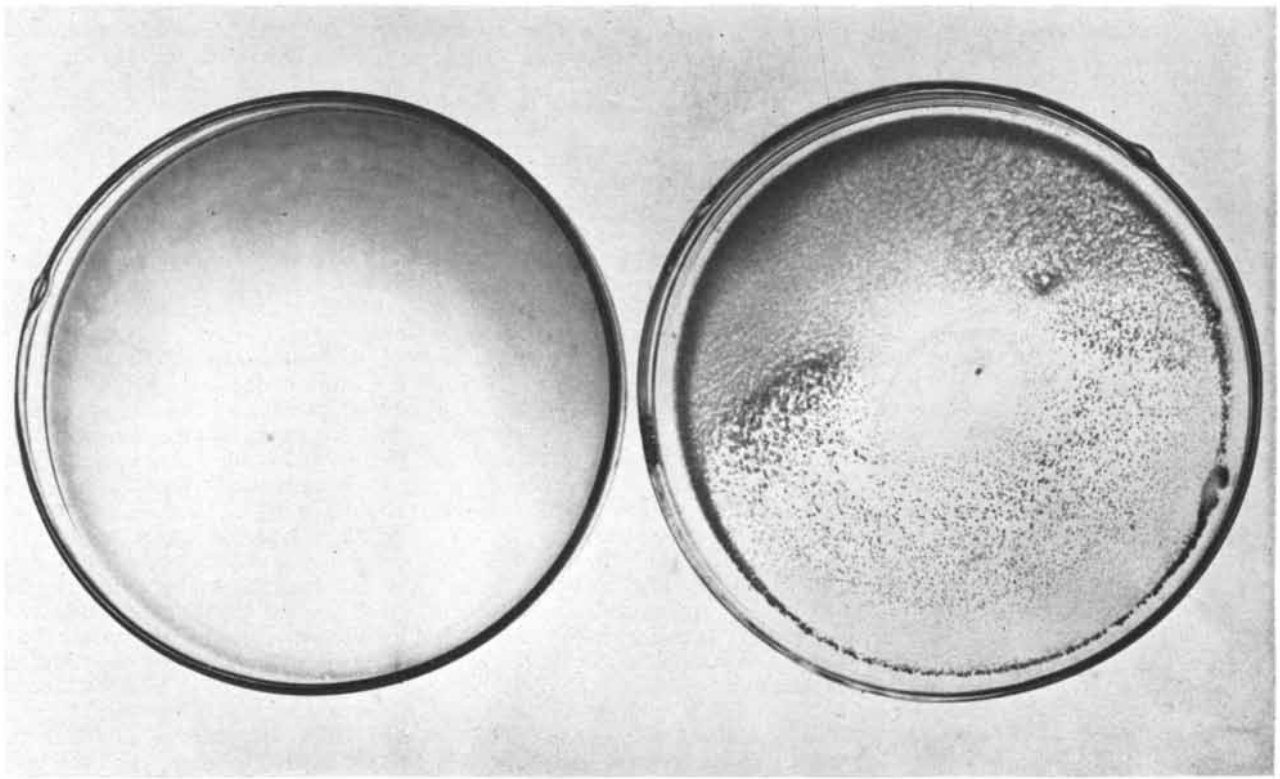
We found, curiously enough, that when a large amount of ordinary influenza virus is mixed with a little of this neuro-flu, the result of the injection is quite different. It might reasonably be expected that with the double infection the mice would probably die just a little sooner than if they had the neuro-flu alone. In fact they usually show no signs of illness whatever. The explanation is that there occurs a type of "interference," in a rather special technical sense of the word, which is well known to virologists. The effect depends on the relative amounts of the two viruses. A mixture of one part of neuro-flu to 10 or 100 parts of ordinary flu is harmless; when the mixture contains equal amounts of both viruses, there is little interference and the death of the animals is delayed only a short time.

An experiment in which mice received a mixture of the two viruses that produced partial interference, with some mice in a group dying and some surviving, yielded another very interesting result. Examination of the viruses in their brains showed that there were not two but three types: neuro-flu, ordinary flu and a third type which possessed several characters of the ordinary virus and the most obvious quality of the neuro-flu, namely its capacity to produce fatal brain infections. The most likely, though perhaps not the only, interpretation of these results is that the third type of virus is a "recombinant" in which the qualities of the other two have been combined.

So far there have been no accounts of any other experiments on this "hybridization" of viruses. For technical reasons it may be hard to find other situations in which the process can be shown. It is unjustifiable, therefore, to say that the conclusions derived from the neuro-flu experiments are applicable to other types of virus. Nor, to be quite honest, do I think that other virologists are yet as convinced as I am that the recombination experiments done in my laboratory in Melbourne have all the significance that I have given them. That is only likely to come when the experiments have been repeated and more deeply analyzed in other laboratories.

With these reservations, our interpretation of the experiments is that influenza viruses multiply in the same fashion as bacterial viruses. For most of the cycle inside the cell, virus as we normally know it is not present. The invading virus particle has given rise to genetic subunits, perhaps a dozen, perhaps a hundred, which for a time multiply virtually independently. We must assume that, as in the case of the bacterial viruses, toward the end of the cycle groups of "genes" come together from this pool of accumulated virus material to reconstruct the infective virus particles. Such a system could account for the various experimental findings: the "disappearance" of virus after the cell has been entered, the production of partial virus, the phenomenon of interference and the appearance of recombinant virus in mixed infections.

These may seem heretical concep-



CLUMPING OF BLOOD is another means of measuring the amount of influenza virus. At left is a dish containing chick red blood cells. At right is a dish of cells to which virus has been added; the cells have formed tiny clumps. The phenomenon was photographed in the laboratory of George K. Hirst, who first discovered it.

tions, and further studies may compel their modification, but there is no possible escape from the general conclusion that viruses are in no sense ultimate particles. They are complex organisms, with a genetic mechanism which has to be thought of as something other than the virus particle as a whole and which seems to be built up of units analogous to the genes of higher organisms.

Their Size and Chemistry

To the layman the most interesting thing about viruses is their smallness. There is a tendency to feel that until you can see something there is no way of studying it. This of course is a complete fallacy. With the electron microscope we can now produce very detailed pictures of influenza viruses and of the bacterial viruses, and every virologist has been excited and delighted by seeing them. We must know what viruses look like to satisfy our curiosity and to provide background for the refinements in the use of electron microscopy which in the future will make it a really valuable technique. But it is fair to say that what is revealed by these pictures has hardly helped at all in understanding how viruses produce the effects that make them so important. At the present time our pictures are only of the free virus particles; for technical reasons it is not yet possible to see what is happening while the virus is multiplying in the cell. Electron microscopists who are interested in viruses are seeking to devise ways in which clear pictures of what is happening in the early stages of cell infection can be obtained. This is obviously not going to be an easy task, but one can feel reasonably certain that it will be accomplished.

What electron microscopy has achieved so far is to show that viruses come in a considerable range of sizes. The smallest, those of poliomyelitis and foot and mouth disease of cattle, are approximately one-twentieth the diameter of the large ones, *e.g.*, those of psittacosis and smallpox. Nearly all appear roughly spherical in the electron microscope, but their true shapes may be distorted considerably by the drying in a vacuum which is an essential part of the preparation of a specimen for this instrument. There is one sharp exception to the rule of spherical shape. Certain types of the influenza and related viruses are extremely long and filamentous, and these are almost certainly an alternate form of the actual infectious virus. In the chick-embryo fluids containing these long forms, there are always large numbers of short and round forms as well. It has not yet been conclusively proved that the long forms will actually cause infection, but they certainly behave just like the small forms in the way they attach to the surface of a red blood cell.

The chemical structure of viruses is in somewhat the same shadowy realm as their physical appearance. With sufficient effort, instrumentation and ingenuity it is possible to obtain milligrams of "pure virus" from the fluids or tissues of infected animals. This can be analyzed by accurate micromethods for its elementary composition—so much carbon, hydrogen, nitrogen and phosphorus—and for the proportions of protein, carbohydrate, fatty materials and nucleic acids. With the new method of paper partition chromatography it is even possible to check the individual amino acids of the protein and the components of the nucleic acids. The results, however, tell us little more than that viruses are built of the same sort of material as other living organisms. Nucleoprotein, the most important component of the chromosomes in higher cells, is always present in viruses in moderately large amount; plant viruses may contain nothing but nucleoprotein.

Unfortunately a truly pure virus is a chemist's dream rather than a biological reality. If our views on virus multiplication are correct, one could never expect the virus particles made in the interior of a disintegrating cell to be free of adventitious fragments of substance from the cell. There is direct proof of this in the fact that influenza virus grown in mouse cells and thoroughly purified still shows by its reaction with an "anti-mouse" serum that there are some

"mouse molecules" as well as virus substance on its surface. Similarly the same virus grown in a chick embryo can be shown to have some chick substance incorporated in it.

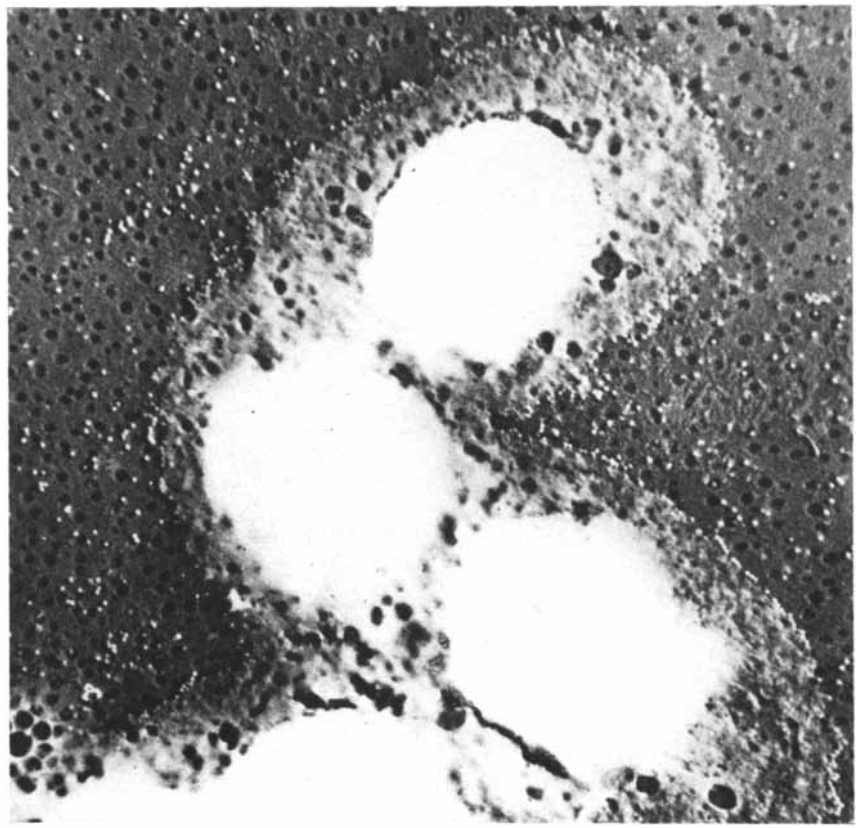
For these various reasons our information on the size and chemical nature of viruses is too meager to be in itself of any current help in understanding virus disease.

Immunity

So far in this discussion of the virus and the cell, the host has played a purely passive role. The virus is the invader, and the effectiveness of its attack, it would seem, depends only on whether its genetic make-up is appropriate to the host cell concerned. Fortunately life is not like that. There is a rule about infectious disease to which I know of no exceptions: Whenever a parasite and its host species have lived together for many generations, they will have found a *modus vivendi* whereby the parasite species survives without producing more than minor damage to the host species. It would be of no advantage to the influenza virus to be so virulent that every human cell could be invaded and every human being killed in some ghastly pandemic. Having murdered its host, the virus itself would perish just as completely. The dramatic epidemic that kills a high proportion of those it strikes will always on investigation prove to be the result of some new development. In the old days of yellow fever in the West Indies the native population appeared unaffected by the disease, while European armies melted away in a few months under its onslaught. The Europeans were intruders into a virtually stabilized biological equilibrium.

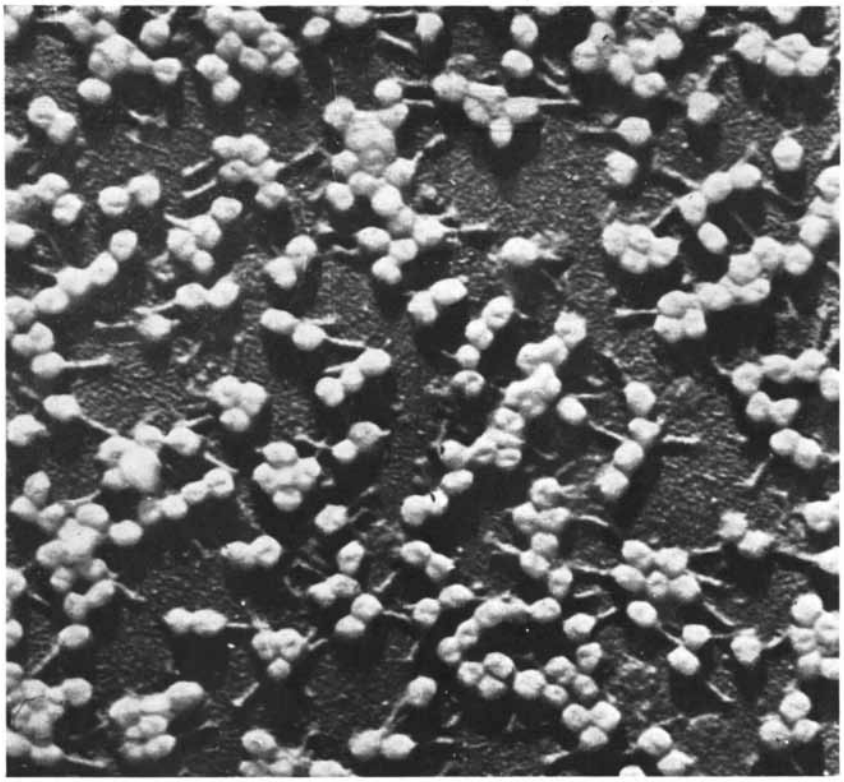
The practical control of a virus disease nearly always depends essentially on obtaining an understanding of the means by which the balance between the virus and the host is maintained in nature and how it can be modified in either direction by biological accident or by human design. In the approach to such an understanding two important related concepts have emerged—"subclinical infection" and "immunization."

A subclinical infection is one in which the infected person gives no sign of any ill effect. In a population attacked by an infectious disease, subclinical infections often greatly outnumber those severe enough to produce unmistakable symptoms of the disease. For example, when a child comes down with a paralyzing attack of poliomyelitis, a careful examination of the rest of the family will commonly reveal that all the other children have the virus in their intestines over a period of a week or two, but they either show no symptoms at all or have only a mild, nondescript illness. Fortunately even a subclinical infection produces

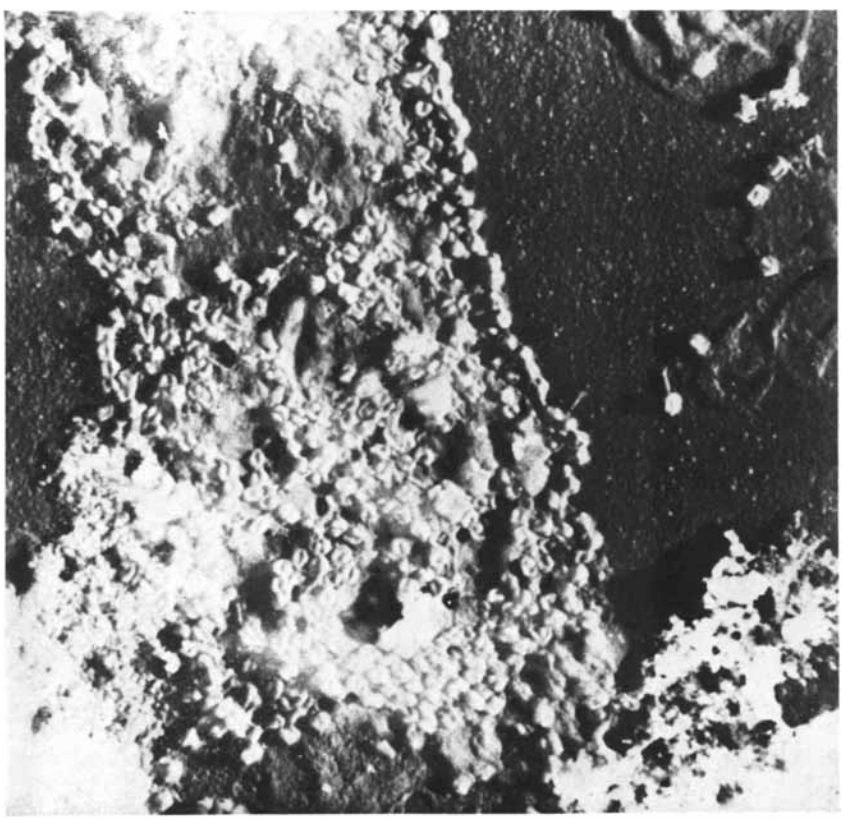


CLUMP OF RED CELLS in the presence of influenza virus is shown by an electron micrograph. This micrograph, which enlarges cells and viruses 5,300 times, was made by F. Heinmets at the University of Pennsylvania.

INFLUENZA A AND B VIRUSES adhere to the dried "ghost" of a red cell to which they were adsorbed. This micrograph, which enlarges the viruses 9,000 times, was made by F. Heinmets at the University of Pennsylvania.



BACTERIAL VIRUSES which infect colon bacilli are revealed by an electron micrograph. This micrograph, which enlarges the viruses 40,000 times, was made by R. W. G. Wyckoff of the National Institutes of Health.



COLON BACILLUS infected by bacterial viruses is almost completely converted to virus particles. This micrograph, which enlarges the viruses 63,000 times, was made by R. W. G. Wyckoff of the National Institutes of Health.

heightened resistance or immunity to the virus for a period after the attack. This capacity of mild or subclinical infection to confer immunity is probably the greatest factor in maintaining a tolerable equilibrium between man and the common virus diseases. The trouble is that viruses are labile beings, liable to undergo mutation in various directions, and a virus that causes only mild infection may evolve into one far more deadly.

Malta and St. Helena

Perhaps the best available example to illustrate this point is the contrast between two epidemics of poliomyelitis that occurred during the last decade, one on the island of Malta, the other on St. Helena. Both were severe epidemics, but one attacked a much wider range of victims than the other. The Maltese epidemic (which began a few months after the siege of Malta had been lifted in 1943) was almost entirely restricted to the youngest children among the island's inhabitants; over 90 per cent of those paralyzed were under five years of age and more than half of these were under two years. On the other hand, the St. Helena epidemic not only involved the youngest children but also hit hard at the older age groups and even considerable numbers of adults.

We can assume that on both islands an unusually virulent type of virus was active and that every inhabitant was exposed to contact with infection. What was the reason for the difference in the results on the two islands? The clue lies in the past history of poliomyelitis in the two places. On Malta a few cases of infantile paralysis, almost wholly among very young children, had been reported each year for as long as accurate medical statistics had been kept. Evidently polio viruses of low virulence had been steadily disseminated among the population for many years. In such a community most of the babies would become infected quite early in life. Since the viruses were not very virulent, only a tiny proportion would be paralyzed. The others would develop a certain degree of immunity which later infections would strengthen. When in 1943 a more virulent polio virus appeared, the older children and adults, who had acquired such immunity, were little affected by the new virus. But among very young babies not previously exposed, the new virus caused a much higher proportion of paralysis than had the earlier mild forms. In other words, the fact that subclinical poliomyelitis had been prevalent on Malta for years, possibly for centuries, had ensured immunity against paralysis for all but the unlucky infants whose first contact with the virus was with an unduly virulent variety.

The poliomyelitis history of St. Helena was quite another story. On that island

there had been no record of infantile paralysis for at least 20 years. It was a "virgin soil" for the virus. None of the adolescents or young adults had any immunity from previous exposure to polio. Hence the new invader struck with paralyzing effect in all these age groups.

The Host's Defenses

Immunity to virus disease was known long before any virus could be handled in the laboratory. In fact, it was from Jenner's early vaccination attempts against smallpox that the whole science of immunity sprang. But then immunology turned almost wholly to bacterial diseases. The toxin-antitoxin approach, which developed from the late 19th-century discovery of the cause and the means of prevention of diphtheria, for many years dominated the outlook of immunologists. In recent years the study of immunity in virus disease has been renewed, and it has profited from the concepts developed in the bacterial investigations.

One cannot claim that there is full agreement about the nature of immunity to viruses, but it is possible to offer a simplified account which most virologists would accept as at least the most convenient approach to understanding that is available at the present time. This interpretation is that all immunity to viruses is mediated through antibody, and that the effectiveness of the protection depends first on the amount and character of the antibody and second on the availability of the antibody to protect the particular cells that are exposed to infection. Antibodies can be described most simply as modified blood-protein molecules which are capable of attaching themselves firmly to the specific virus or other invading organism that provoked their production by the body. If a sufficient number of antibody molecules can attach themselves to a virus particle, they have a blanketing effect which effectively prevents the virus' attachment to the host cell and its multiplication within the cell.

Antibody appears in the blood a few days after infection and reaches a peak in two to three weeks. The body continues to produce antibody at a slowly diminishing level long after recovery—in some diseases, such as measles and yellow fever, for the whole of life. Immunity is long-lasting only against those diseases in which the virus must pass through the blood at some stage before it produces symptoms. The explanation, in general, is as follows: After any virus infection, the antibody produced in response to it is always concentrated most abundantly in the blood. In a disease such as measles, where the virus must pass through the blood, the large amounts of antibody there waylay any virus in a second invasion and render it inert before it has a chance to create any symptoms of illness. The virus of a disease such as influenza, which does not have to pass through the blood but spreads from cell to cell over the surface of the air passages in the respiratory system, has an easier time. The concentration of antibody here is always less than in the blood, and it soon declines to an amount insufficient for protection. Hence the immunity that follows an influenza infection is less complete and less lasting than that in a disease where the virus must run the gamut of the blood.

Artificial Immunization

To return to the problem of how a tolerable equilibrium between man and a common virus is maintained, the situation can be summarized as follows: The first contact with the virus normally takes place in childhood. How early it will occur depends on how prevalent is the virus and how effective are the social barriers against its spread, such as cleanliness and good housing. The standard virulence of the virus is low, and young children as a rule recover after a mild illness or no illness at all. This induces an immunity not only against virus of normal or low virulence but also against the occasional type that has undergone mutation to higher virulence. The process will never be completely effective. As long as the common virus diseases (measles, influenza, poliomyelitis) persist, there will be epidemics in which some patients will require all the help the physician can provide. But under present-day conditions the great majority of people pass through childhood and middle life with no more than trivial episodes of infectious disease. They have not escaped infection, but by the sequence of subclinical infection and immunization they have been kept from even knowing of its occurrence.

It is against virus diseases not commonly present in their own communities that people most need the protection of artificial immunization. Men having to work or fight in the tropics of Africa or South America must be immunized against yellow fever. Smallpox, still prevalent in many parts of the globe, may enter any country, so vaccination is a necessity for any traveler and desirable for all. In these two instances immunity is produced by procedures which very closely imitate the natural process of subclinical infection. The immunizing agent is a living virus, a variant of the virulent form which can be relied upon to produce no more than trivial symptoms. If its safety can be assured, this is the most effective type of immunization. But in many cases it is not possible to produce a variant that is both effective and safe. The only available method of immunization against such viruses is to inject relatively large amounts of killed virus. On the whole this is not a particularly satisfactory method, and the only human disease against which it has proved reasonably effective is influenza. Provided the proper type of virus is used in preparing the vaccine, and provided the immunizing dose is given not too soon before the epidemic, about 80 to 90 per cent protection can be expected.

The Hardest Question

From its very nature virus research, like bacteriology in general, has tended in the past to concentrate on medical and veterinary problems. It will probably always be carried on against a background of its significance for medicine. But if one looks around the medical scene in North America or Australia, the most important current change he sees is the rapidly diminishing importance of infectious disease. The fever hospitals are vanishing or being turned to other uses. With full use of the knowledge we already possess, the effective control of every important infectious disease, with the one outstanding exception of poliomyelitis, is possible.

Today the most intellectually exciting aspects of virology are not directly concerned with medicine. As I see it, the main interest of the virus to biology now is the possibility of using it as a probe in the study of the structure and functioning of the cell it infects. In many ways the cell is the center of life, the unit from which all but the very smallest organisms are built—ourselves included. All the biological sciences, with biochemistry and genetics in the lead, seem to be converging to attack the central problem of cellular structure and function. In this endeavor the detailed study of the interaction between the virus and the cell promises to be very fruitful.

The very smallness and simplicity of viruses have a special attraction to the biochemist bold enough to look for an answer to the hardest question he can ask: How is the specific pattern of living chemical structure reproduced within the cell? The answer to that will be more likely to come from the study of plant viruses than from any other source. For similar reasons geneticists are attracted to the possibilities arising from the investigations of the recombination of genes in bacterial viruses, now also becoming visible in some of the animal viruses as well.

Microbiology is today the queen of the biological sciences. It can provide biologists with work and pastime and reward for many generations to come.

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