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Scope and Method of Study: The library was searched for information about basic research and how the masses of the people need to be informed of the many benefits to humanity through continuous research.

Findings and Conclusions: We can no longer afford to allow scientific genius to remain idle, nor can we afford to concentrate all our attention upon the physical sciences for the war against diseases is equally as important. The problem, however, goes beyond the mere provision of financial aid to our scientists. To adapt ourselves to the spirit of this century, we must reassess our whole philosophy and our sense of values. Just as the Stone Age was characterized by the use of stone, so our era will undoubtedly go down in history as the Age of Research.

The impact of antibiotics and other wonder drugs on society is immeasurable. These life-saving drugs that were laboratory curiosities are now being used for agriculture, food preservation, and to treat plant diseases.

Experimentations with bacteriophages shed new light upon the constitution and mode of action of the viruses. Through the study of the viruses, tissue culture propagation <u>in vitro</u> was discovered. This made possible the preparation of the poliomyelitis vaccine in kidney cells.

It is possible through continuous research and the coordinated efforts of our many scientists to gain control over many of the diseases that are now plaguing mankind.

ADVISER'S APPROVAL [signature]

BASIC RESEARCH IN THE ANTIBIOTICS AND VIRUSES
AND RESULTING BENEFITS TO MANKIND

By

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BASIC RESEARCH IN THE ANTIBIOTICS AND VIRUSES AND RESULTING BENEFITS TO MANKIND

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CHAPTER I

INTRODUCTION

We have come a long way since colonial days when practically all our scientific knowledge was imported from the older established countries of Europe. The problem is different today. It is a matter of providing adequate support, organizational as well as financial, for the growth of research and the educational system upon which it depends. All parts and groups of the nation and world stand to gain, materially and spiritually, from the encouragement of scientific work. It is the opinion of the writer that the growth of research cannot be forced, but takes place naturally where there is open discussion and the sharing of ideas.

The purpose of this paper is to discuss the necessity of basic research and how its development has benefited mankind.

The procedure followed was:

1. In Chapter II - Basic research is explained and the steps necessary to improve our national effort in promoting basic research are outlined.

2. In Chapter III and IV - Examples are given of great achievements that were primarily due to basic research. However, further development of these discoveries was necessary before the finished products were available for chemotherapeutic use.

It is the hope of the writer that this paper will serve to create a better insight into the significance of research for those who will read it.

Scientists will continue their activities and the world will continue to change. Their efforts must be aided; for though what they do may yield dangers, the dangers are far greater if they do less. And since what they do affects the world--you, me, our community, our country and the world--we should strive to understand that continuous research is needed in order to produce those things that man needs to improve his health, his comfort, his happiness and his security.

CHAPTER II

BASIC RESEARCH

Not only will men of science have to grapple with the sciences that deal with man but - and this is a far more difficult matter - they will have to persuade the world to listen to what they have discovered. If they cannot succeed in this difficult enterprise, man will destroy himself by his halfway cleverness. (Waterman, 1957).

Science has played an important role in the rise of the United States from frontier land to world power. The growth of our scientific effort parallels the growth of our strength as a nation. The physical means to conquer wilderness, control natural forces, increase the length and hopefully the enjoyment of life, achieve national security -- these things have come largely from ideas which observation, measurement, and other laboratory arts have developed into useful methods and machines at home and abroad. (Irving, 1949).

Science is a continuous advance to new frontiers. We recognize the adventure of life on a geographic frontier, as in the old West in America; the laws to be obeyed are not clear, the unexpected happens at every turn, the conflicts between "good" and "evil" forces - between order and anarchy -- become fierce. But out of such conflicts new nations arise and new social structures are built. (Hutchings, 1959).

The frontiers of the mind offer analogous adventures. There, too, the unexpected is always found. The conflict between "good" and "evil" is analogous to the conflict between knowledge and ignorance. And out of this conflict a new era of human understanding will some day be built, which may intimately affect the structure of our own society.

The geographic frontiers are disappearing. But the frontiers of the mind are never-ending. Ignorance remains an infinite, unconquered wilderness, and the adventure of this conquest will mount in interest as time passes.

Research of today produced both the lifesaving drugs and the destructive weapons of tomorrow. Its outcome will affect everybody, and in a democracy whose people decide how wealth shall be distributed everybody shares the responsibility of developing the nations scientific potential.

Bridging the gap between the scientist and the general public will not be easy. The former will have to learn to translate his own problems into a language meaningful to the layman; the latter will have to realize that, however simplified, the essence of basic research cannot be assimilated without mental effort. (Hutchings, 1959).

What is basic research? Charles E. Wilson, (Selye, 1959), defined it as what you do "when you don't know what you're doing," a sarcasm presumably intended to justify the inadequacy of financial support for basic research.

The writer is inclined to define basic research as the study of natural laws for their own sake, irrespective of immediate practical application -- with emphasis on the qualification "immediate".

The only kind of research usually designated as "basic" is true "discovery". What follows is "development". The former kind is basic, or fundamental, precisely because every other kind of research develops from it. It strikes us as impractical and the work involved as haphazard, because wholly original observations cannot be planned in advance, to do so the observation would have to be anticipated on the basis of previously known facts, and hence could not wholly be original. That is why most of the completely new leads are accidental discoveries made by men with the rare talent of noticing the totally unexpected. These form the basis of all premeditated research projects - the kind we call development. (Selye, 1959).

We regard it as proper and just that the patronage of science by society is in a large measure based on the increased power which knowledge gives. If we are anxious that the power so given and so obtained be used with wisdom and the love of humanity, that is an anxiety we share with almost everyone. But we also know how little of the deep new knowledge which has altered the face of the world, which has changed -- and increasingly and ever more profoundly must change -- man's view of the world, resulted from a quest for practical ends or an interest in exercising the power that knowledge gives. For most of us it has been the beauty of the world of nature and the strange and compelling harmony of its order that has sustained, inspirited, and led us. That is as it should be. And if the forms in which society provides and exercises its patronage of science leave these incentives strong and secure, new knowledge will never stop as long as there are men. (Lessing, 1954).

We must consider how we as a nation may provide the circumstances which encourage the sound and creative development of science.

No one man, no one group, no one agency of government of society, is wise enough, imaginative enough, or broad enough usefully to dominate national decision about the support of science.

It is necessary that in order to improve our national effort in basic research, certain steps should be taken. The first is to establish conditions more favorable to the continued growth of basic research and the second is to achieve a greater flow of funds for basic research. The following suggestions were made by Lessing:

CORPORATE GRANTS

Industry is encouraged to increase the dissemination and exchange of basic research information and to foster - through trade association or other arrangements - cooperative industrywise undertakings in basic research.

Outlays for the training of basic research scientists at universities, like certain other research - and - development costs might be made tax deductible as business expenses. Such deductions should be allowable whether or not the training is essential to the performance of special duties, for the expenditures are essential to improving the scientist's competence in research, and therefore should be regarded as a charge to the research program. (Waterman, 1957).

INCREASED PUBLICATIONS

It is in the national interest that all results of basic research, wherever it is done, be published promptly. This applies to basic research in industrial laboratories as well as laboratories of universities or government. The view is widely held that until the results have been published research has not been carried to completion, for its value can develop only as the new knowledge is widely disseminated. Publication is the vehicle, and the cost of publication is an essential part of the cost of research. (Waterman, 1957).

NON-PROFIT ORGANIZATIONS

Research Institutes: Such organizations may be justifiably expected to put more emphasis on basic research. It has been suggested that federal-tax exemption privileges for non-profit research institutes be modified, along lines of more precise distinction between research performed for the restricted use of clients and research freely and publicly dedicated to society.

Private Foundations: Less of the private foundations 1953 funds was spent for science on basic research than was spent in 1939. The shift away from basic research reflects foundation efforts to aid in the study of pressing public problems. (Waterman, 1957).

It is neither appropriate nor desirable that the government try to deflect the private foundations from their avowed interests and approaches. But federal agencies supporting scientific research should exchange information with these organizations on a continuous basis. The objective is to discover and emphasize fields where basic research is particularly needed.

Professional Societies: Scientific and professional societies, journals and other publications bring much new knowledge to the attention of scientists. Since industry may be choice or indifference fail to publish an appreciable proportion of its basic research, the societies might take an active part in encouraging industrial scientists to submit more papers on basic research studies. These papers could be significant additions to the flow of information in many scientific fields. (Waterman, 1959).

Increased Support by State Governments: Generally speaking, state legislatures tend to be conservative in supporting basic research. But it appears that some of them at least would be willing to increase their support, if the federal government furnished supplementary funds. (Waterman, 1957).

Federal Support: The conduct of basic research would be stimulated if the federal government increased its support of basic research at institutions of higher learning, particularly at universities with graduate schools. The increase might properly reach the point where total funds from all sources were adequate to support fully the competent research workers in all departments who were available and wished to do basic research. The institution, of course, would determine availability.

Physical Facilities: The increasing intricacy of problems needing study, and the depth of knowledge of modern experimental methods of attacking them, call for apparatus and instruments and facilities that are exceedingly complex and costly. Much apparatus must be specially designed and constructed.

As a general principle, federal support to physical facilities should require reasonable financial participation on the part of the institutions concerned, the degree of federal cost participation resting upon mutually satisfactory arrangements. (Waterman, 1957).

Whatever the volume and sources of support we must, in the end, look to our universities for the imagination and wisdom that will secure the maximum benefit to science. (Weaver, 1957). The importance of the universities, and colleges to the nation's advancement in basic research, and hence to the nation's progress through the benefits of research, cannot be rated too highly. These institutions, therefore, have a responsibility, generally acknowledged as one of their traditional functions, of maintaining high productivity of their research scholars and excellence in their scholarship.

Years ago it was considered obvious that basic research was the business of a university, in the non-commercial sense that professors received a modest and decent chance to live, and that they repaid society by the combined service of teaching and research. Professors in those days, considered that society was giving them a marvelous privilege: the opportunity of earning a living by doing what they most wanted to do. It was moreover, an essential and wonderful part of this arrangement that the decision as to what research they did was left to the free choice of the indicidual professors. (Weaver, 1957).

Nowadays research continues to be the business of universities, but there is a real danger that the individual scientists may be losing sight of their basic purpose. University salaries are so poor that it has become necessary, disgracefully necessary, for professors to supplement their income by summer earnings. To balance the family budget many professors now work on a summertime research project, or worse yet, in order to get a favorable reply to an application he uses projects which will fit into some one else's ideas of what is worthy of support. (Weaver, 1957).

Large numbers of the public, the polls tell us, regard a scientist as an unselfish saint if he wears a white coat and has a stethescope, a magician if he is looking intently into a test tube which glows in the dark, and a potentially menacing creature if he is manipulating the controls of a nuclear reactor. As a nation we demand science, but we both over and underestimate it. We continue wrongly to identify science and technology, and we largely fail to recognize that the most important aspect of science is that it gives to man a beautiful rich and deep understanding of the world in which he lives. (Weaver, 1957).

In reorganizing our approach to the support of science we need not set aside utilitarian objectives. Goal orientated developmental research is exceedingly important; frequently it asks questions that inspire fruitful developments in basic research. But society must learn to recognize the importance of the able scientist who is motivated by pure curiosity. The encouragement of science will be disastrously narrow if it does not offer rewarding careers to these men. (Waterman, 1957).

If our society is to encourage creativity in science, we must strive to learn more about science, to appreciate science in an intelligently discriminating way, and thus come to understand the essential role played by the apparently impractical drive of pure curiosity. Society must assure our universities of sufficient basic support so they can offer, to competent and creative scholars, a decent, stable, and honored opportunity to think.

In the following chapters, the writer will endeavor to give examples of great achievements that were primarily due to basic research. However, further development of these discoveries was necessary before the finished products were available for mankind's use.

CHAPTER III

ANTIBIOTICS

"As a man is a creature of the earth, bound to the universe and all its forces; as he gains his ills from the earth, so, too may he draw his balms, his remedies and his cures from the earth." (Sokoloff, 1941).

Millions of people have died in epidemics of scarlet fever, typhoid, influenze and other infectious diseases because man was ignorant of the remedies for these diseases. However, gradually, our knowledge of bacteria began to grow, through studies of their weaknesses and limitations, until now we have gained knowledge of many of the germs which cause disease. These diseases can be diagnosed by medical science only because of continued basic research.

Many centuries ago, man was little troubled by infectious diseases but with the development of civilization the power of minute microbes to take human lives steadily increased. The history of the human race interpreted in the light of medical science has been a never-ending war against germs. (Kaefer, 1943).

A really great achievement of basic research was the observation by Sir Alexander Fleming that penicillin can kill varieties of disease producing microbes, at dose levels tolerated by man. This has enabled other investigators to discover many useful drugs, derived like penicillin, from molds. It is surprising to find that molds, which we regarded as contaminators, have a curative value. Many bacteriologists had seen that cultures of microbes were spoiled when exposed to molds, but all they concluded was that molds must be kept out of such cultures. It took a stroke of genius to see the medicinal promise of the basic observation. (Selye,1959).

Antibiotics, in the broadest sense, are substances produced by living organisms, which in small amounts, can inhibit the life processes of other microorganisms. The word "antibiotic", in a strict sense, is used for a chemical substance, of microbial origin, that has the capacity to inhibit the growth of bacteria and other microorganisms and even to destroy them. Antibiotic substances are of general interest in chemistry and biology, but their greatest impact on science has come about through practical applications in Medicine and Agriculture. (Burkhalter, 1959).

THE HISTORY OF ANTIBIOSIS

It is well known that antibiotics are not the first effective, specific drug used in medical science for conquest of human disease. Beneficial remedies of plant origin were introduced into Europe for use against malaria and amoebic dysentery as far back as the 17th century. There was, at that time, no underlying rational or basic conceptual scheme of chemotherapy. Cinchona bark and ipecacuanha rested on their own merits as simple remedies, produced in nature and discovered through early empiricism. (Burkhalter, 1959).

A century ago Louis Pasteur (1857) observed that onion juice inhibits growth of lactic acid bacteria without affecting certain other kinds of microorganisms. A little later, Pasteur (1877), Babes (1885), Garré (1887), and others noted how certain common bacteria could stop the growth of the anthrax bacillus and other kinds of microbes. It was even suggested by these early microbiologists that some day antagonistic properties of microorganisms might be used to combat infectious agents of disease. The scattered data on microbial antagonism in the late 19th century were not integrated into any formal scientific doctrine. Had it been an earlier crystallization, the antibiotic concept might have directed research sooner toward desired goals. (Burkhalter, 1959).

Antibiotic agents are, in the surest sense of the word, chemotherapeutic agents. The term is derived from the word "antibiosis" which was first used by Vuillemin in 1889, to describe the phenomenon where one organism is in opposition to the life of another. Actually, several antibiotic preparations, such as pyocyanin, pyocyanase, prodigiosin, and mycophenolic acid, were prepared before 1900, but unfortunately none of these could be developed for use in chemotherapy because of their ineffectiveness and toxicity. Antagonistic relations among microorganisms were not completely forgotten after 1900. According to a monograph on microbial association published by Papacostas and Gaté in 1921, an inhibition resulting from the association of two microbes "in vitro" is called "Antibiosis" ("anti - against - bios - life"). (Irving, 1949). Progress in the field seems to have rested, in the first quarter of the 20th century, with recognition of the important but generally overlooked fact that microbial antagonism does indeed exist.

PENICILLIN - THE WONDER DRUG

The next great and independent advance came in 1929 through chance observation. Dr. Alexander Fleming (later Sir Alexander Fleming) was a bacteriologist in St. Mary's Hospital in London. He was using a culture of the pathogenic organism, Staphyloccus Aureus, in some of his studies, when a stray, air-borne spore of a mold, fell into the open Petri dish which contained this culture. Several days later, when Dr. Fleming had occasion to return to the dish, he noticed a very remarkable phenomenon. A mold was growing in the middle of the staphylococcus culture and, as a result, the colonies of bacteria were gradually disappearing. Obviously, the culture was of no further value for its original use, and many investigators would have discarded it. Dr. Fleming, however, noticed that the destruction of the staphylococcus was a result of the contaminating mold and decided to look further into the matter. Successive experiments proved that in all cases the mold apparently secreted a material which was antagonistic not only to the original staphylococcus organism, but also to others in the same general classification. Fleming knew that the mold which caused the disappearance of the bacteria was a "Penicillium" and therefore named the substance which was apparently secreted "penicillin". The mold was later identified as Penicillium notatum. (Irving, 1949).

ANTIMICROBIAL SPECTRUM

Although it has been a number of years since penicillin was introduced into therapy and although many other antibiotics have since been introduced, it still maintains a preeminent position in spite of certain limitations. Its major limitation is its restricted antimicrobial spectrum, for it is a highly selective agent, being inhibitory against most Gram-positive cocci and certain other groups of bacteria but almost without effect on Gram-negative bacilli and the acid fast organisms. Gram-positive organisms sensitive to penicillin are: Streptococci, Staphylococci, Diplococcus pneumoniae, Neisseria gonorrheae and meningitidis and Treponema pallidum to name a few. Also, the Psittacosis virus and the Lymphogranuloma inguinale virus.

MODE OF ACTION AND USES

Penicillin is not only bacteriostatic but it is also bactericidal (lethal to bacteria) and in some species it induces lysis (dissolution of the bacterial bodies). It acts only on bacteria when they are actively growing and dividing. In concentrations too small to arrest total growth, it appears to hinder cell division, so that misshapen forms appear - giant forms or long threads instead of the normal short bacilli. The exact mode of action is unknown, but it seems likely that penicillin interferes with the enzymic action involved in the metabolism of mucleic acid of the growing microbe. (Encyclopedia Britannica, 1961).

At first it was thought that there was only one penicillin, but it was later discovered that the mold could produce at least four penicillins differing slightly in their chemical structure. These have been named in the United States, F, G, X and K, and in Great Britian 1, 2, 3, and K. (Encyclopedia Britannica, 1961).

Penicillin as an aerosol is a useful method in the home treatment of patients with chronic pulmonary infections. By using this method, penicillin is absorbed in the lungs.

In the two most common venereal diseases, gonorrhea and syphilis, penicillin is the most effective agent. Gonorrhea can be cured in 98 per cent of cases within twenty-five hours when 150,000 units are given in divided doses over a twelve to fifteen hour period.

In syphilis, it is now well established that penicillin will cure 70 to 75 per cent of the cases when it is given in the primary or secondary stage of the disease. It is usually given in divided doses over a period of four to sixteen days. It has been extremely effective in preventing congenital syphilis when syphilitic mothers were treated during pregnancy.

For the treatment of all forms of bacterial meningitis due to Gram-positive microorganisms, penicillin is one of the main drugs of choice.

Penicillin has also reduced the fatality rate of pneumococcus pneumonia. It is being used to treat hemolytic streptococcus infections as well as a number of relatively uncommon diseases such as anthrax, actinomycosis and gas gangrene.

In staphylococcus infections in various parts of the body, penicillin has brought about an extraordinary reduction in the fatality rate and has considerably decreased the total days of illness. (Irving, 1949).

RESISTANCE OF SOME MICROORGANISMS

Organisms are assumed to be resistant to penicillin if more than 1 unit/ml. of penicillin is required to inhibit them. (Robinson, 1953). Perhaps the most acute problem that our doctors are now facing is the problem of antibiotic resistance. It has been known for some time that certain disease - producing organisms can become resistant to the action of antibiotics. An extremely serious problem now exists in many of our hospitals with a highly resistant form of Staphylococcus aureus. This organism is always present in hospitals because it normally is present on the skin of all humans. Because of the almost constant contact that it has had with the major antibiotics, certain highly resistant "hospital - strains" have been produced. Hospital personnel, because they are humans, act as "carriers" and can therefore infect patients with this resistant form of the organism. (Day, 1959).

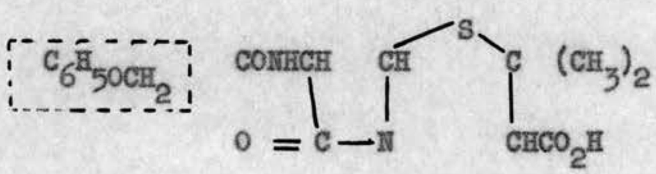
Mutations are responsible for development of resistant forms of Staphylococcus aureus. The step-wise increase of resistance to penicillin is explained by assuming that mutation in several genes are instrumental in the process. The steps are uniform and 1st step resistance is low. This is explained by assuming that mutation in equally potent genes are effective in producing resistance. (Demeric, 1948).

PENICILLIN SYNTHESIS

The chemical synthesis of penicillin which for years has been one of the most baffline problems in chemistry, has been accomplished by John G. Sheehan and K. R. Henery-Logan. Ten new kinds of synthetic penicillins were tested for possible medical use. While the new chemical method was not economical enough to compete with the established fermentation process by which penicillin is derived from molds; it was hoped that the new forms would prove effective against disease organisms resistant to natural penicillin such as Staphylococcus aureus and against a wider variety of disease causing bacteria. (Sheehan, 1957).

The penicillin molecule is not very complex, but it is extremely fragile. The principal problem was to devise methods of building the molecule without breaking it down as rapidly as it was put together. The reactions are largely carried out at neutral pH, and the last five steps are done at or below room temperature.

The formula of synthetic "natural" penicillin: (Sheehan 1957).



Penicillin is indeed a benefit to humanity but the search for newer antibiotics did not stop with its discovery. Another discovery came just at the right moment when many persons thought that there would be no more potent antibiotics. In 1939 René Dubos demonstrated two new crystalline antibiotics, gramicidin and tyrocidin were produced by the bacterium, Bacillus brevis. Therefore, it appeared that there might be many more antibiotic substances in nature. (Burkhalter, 1959).

The next great advance came with the advent Streptomycin, then came the broad-spectrum antibiotics such as chlormphenicol and the groups of tetracyclines, Nystatin, Kanamycin and the Macrolides to mention a few. Many hundreds were "discovered" but to be very useful in medicine an antibiotic must be more injurious to pathogenic organisms than to the human body. The drug should be comparatively stable, easily absorbed, decisively active against living agents of disease, and without harmful side effects. When its work is done it should disappear from the patient's body. Several antibiotics have measured up to these rigorous requirements. (Burkhalter, 1959).

SOURCES OF ANTIBIOTICS

Some species of microbes produce several different kinds of antibiotic compounds, and it is also true that a given compound may be synthesized by more than one species of mold or actinomycete. The rapid growth of the list of new compoinds comes about as a result of intensive efforts to find drugs that may be useful against special pathogens and against disease agents that have become resistant to the older antibiotics. The majority of the new compounds seem to come from fresh isolates of the genus Streptomyces. Common sources of antibiotics are also found among the molds and bacteria that live abundantly in soils, composts, and other places. Larger organisms, such as mushrooms, and lichens, also produce antibiotic substances. In many flowering plants and in some coniferous trees, antimicrobial compounds of unusual nature or with chemical structures not unlike those of some of the mold antibiotics, are known to occur. A few kinds of algae elaborate antibacterial substances. Some kinds of corals produce compounds which strongly inhibit the acid-fast group of bacteria, to which the tuberculosis germ belongs. (Burkhalter, 1959).

RECENT TRENDS

A noticable recent trend is that of increasingly extensive manufacture of antibiotics for uses other than in human medicine. Large quantities of antibiotic drugs are used in veterinary medicine. Sometimes, however, there may be some carry over of antibiotics from treated cows into the milk supply where it may interfere in the microbial conversion of milk to cheese,(a bacterial process), or they may cause death or extreme pain, discomfort due to allergic reactions in sensitive persons.

Antibiotics are also being used in agriculture as supplements in animal feeds. Practically all commercial poultry feeds contain low-level supplements of antibiotics such as penicillin, the tetracyclines, erythromycin or bacitricin. Antibiotic-fed pigs, poultry, horses, and mink grow faster, require less feed, and show lower mortality rates than those not fed antibiotics. This results in tremendous economic gain to the farmer. (Burkhalter, 1959).

Antibiotics are being developed for a variety of other uses; these include everything from sprays for the arrest of bacterial and fungus diseases of plants to agents for increasine fecundity in cattle, agents for short-term preservation of foods, and many other uses. Preparation for slaughtering steers in some countries includes injection of antibiotic preservatives; these are carried into the tissues of the animal, so that later the meat can be hung in warm rooms to bring about tenderization without spoilage.

It is difficult to say how great have been the contribution of antibiotics to public health. It seems quite clear, however, that marked reduction in mortality from many infectious diseases has occurred during the years since science provided society with "miracle drugs". Ponder, but not too long for it is of too great magnitude, the impact on politics, knowledge and economics of people who are now alive only because of a basic observation made in a small laboratory by a teacher-scientist.

CHAPTER IV

VIRUSES

DISCOVERY OF THE BACTERIOPHAGE

The smaller bugs have smaller bugs which live upon them and bite them. - - - Adage

The discovery of the existence of bacterial viruses has opened new fields not only to the bacteriologist but to all biologists who are interested in the fundamental nature of living things. Since the bacteriophage shares many properties with animal and plant viruses, it is assumed that studies on the bacterial virus will grant a better understanding of those viruses pathogenic to animals and plants.

Knowledge of the bacterial virus began with the observations made by Dr. F. W. Twort, an Englishman, and R. F. d'Herelle of the Pasteur Institute in France. (Rodriques, 1945).

Dr. Twort, in 1915, described a phenomenon in which the colonies of staphylococcus cultures obtained from glycerinated calf vaccinia became glossy and transparent. (22). He was able to transmit this change from one culture of the susceptible organisms to another by placing on an inoculated agar slant a drop of a highly diluted filtrate from a suspension of an earlier growth that had undergone the degenerative change.

In 1916, Dr. d'Herelle (1926), working independently of Twort, observed that filtrates obtained from the feces of patients convalescing from bacillary dysentery actually dissolved the cells of the susceptible bacteria. He was able to demonstrate the transmission of the lytic agent. In a prolonged series of cultures of the susceptible bacteria, he added the filtrate obtained from the preceding one after lysis had occurred. The name that he applied to the lytic agent, "bacteriophage", has come into general use, familiarly shortened to the diminutive, "phage"; and the view maintained by him was that this agent is a filterable virus, pathogenic for bacterial cells. There are, also, earlier records of curious happenings and appearances in bacterial cultures as instances of this lytic change. Workers observed clear areas in the midst of confluent growth of the bacteria responsible for a disease of locusts as early as 1909. (d'Herelle, 1926). However, in none of these cases was the nature of the process studied in any detail, nor was its transmission by bacteria-free filtrates demonstrated.

REPRODUCTION OF BACTERIAL VIRUSES

The two major constituents of bacterial viruses are proteins and nucleic acids. As a general rule, the protective coat of viruses is made of protein and the active part is the nucleic acid, DNA in almost all instances.

The tail is responsible for the virus's ability to infect bacteria and for governing the rate of its multiplication and which must inject the hereditary features of the virus into the bacterial cell. The tail contains an anzyme, which probably has the function of opening a hole in the cell wall of the bacterium. (Pollard, 1954).

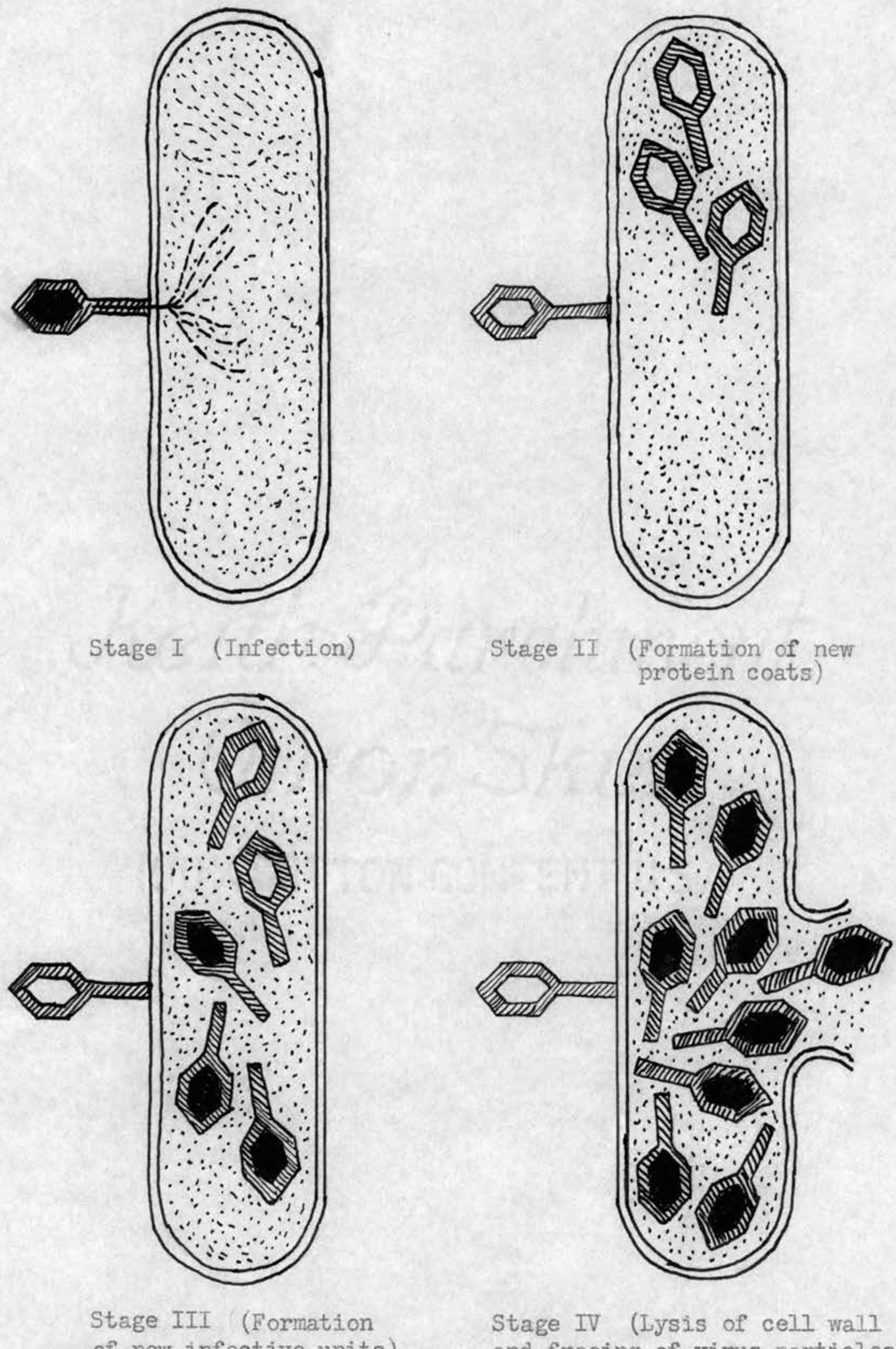
Reproduction of bacterial viruses occur in four stages which are as follows:

Stage 1. Infection. The virus particle attaches itself, by the tail, to the cell wall of the bacterium. The nucleic acid core of the virus empties into the bacterial cell; the protein coat of the virus remains outside. (Figures 1 and 2).

Stage 2. The dark period. This occurs about 10 minutes later. The viral nucleic acid has begun to multiply within the bacterial cells, and has induced the formation of new protein coats. The protein coats contain no nucleic acid; there are no infective particles, not even the particles that caused the infection.

Stage 3. The rise period - about 20 minutes after infection - now some of the protein coat contains nucleic acid; the first infective particles of the new generation have made their appearance within the bacterial cell.

REPRODUCTION OF BACTERIOPHAGES



Stage I (Infection)

Stage II (Formation of new protein coats)

Stage III (Formation of new infective units)

Stage IV (Lysis of cell wall and freeing of virus particles)

FIGURE 1

REPRODUCTION OF BACTERIOPHAGES

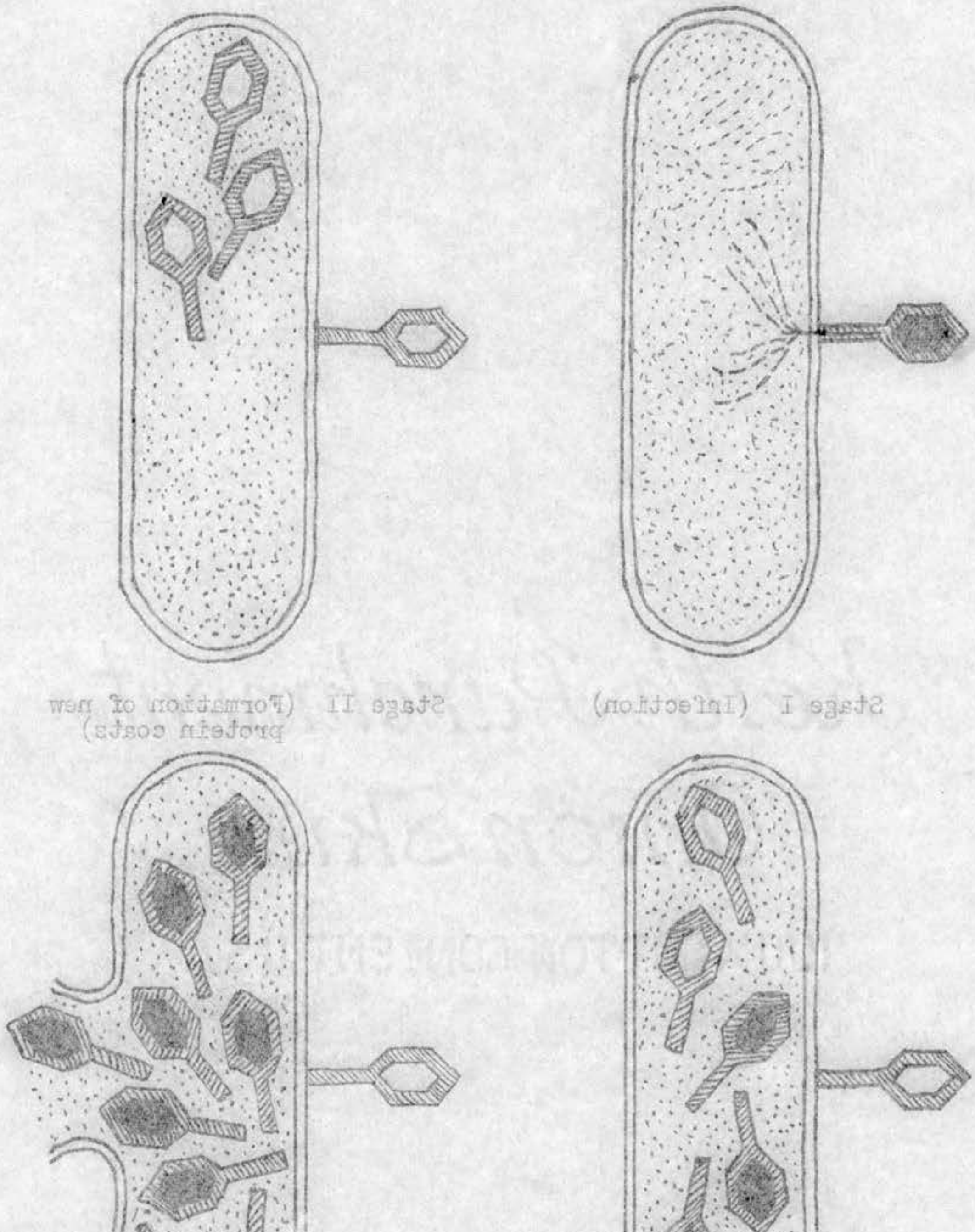
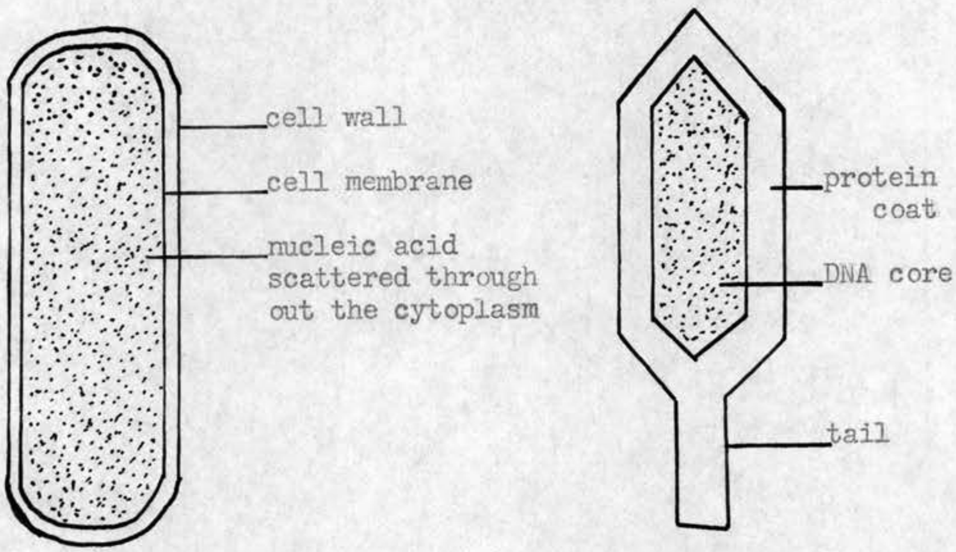


FIGURE 1

FIGURE 2

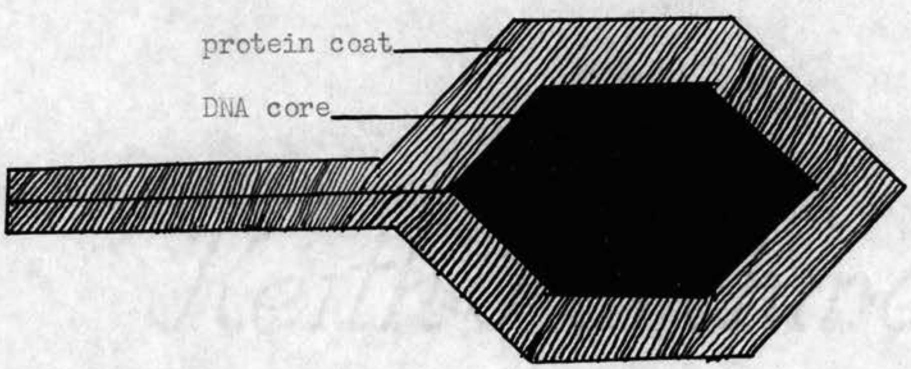
PARTS OF THE BACTERIAL CELL AND VIRUS PARTICLE



Bacterial host cell   Virus particle

FIGURE 3

BACTERIAL VIRUS OF THE T_2 STRAIN



as therapeutic agents. There is no doubt in the mind of the men of science that the bacteriophage has greatly enhanced the study of viruses.

THE NATURE OF THE BUG

Poliomyolitis, influenze, and the common cold - probably the three infectious diseases of most interest to the average person - are all caused by viruses. So are small pox 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 their control. (Burnett, 1951).

A virus can be defined as a microorganism, considerable 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 virologists's problem from that of the classical bacteriologist. A bacterium 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.

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. Second the experimental host must show some sign or symptom that will allow the experimenter to know when it is infected. (Burnett, 1951).

HOW A VIRUS ATTACKS THE CELL

From large numbers of experiments in many laboratories we have a fairly clear picture of the process by which a 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 (substrate). 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. (Burnett, 1951).

The points on the cell surface to which the virus attaches are spoken of as receptors, and the complex carbohydrates of which they are composed belongs to the class called mucins or mucopolysacchrides. These are 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. (Burnett, 1951).

THE HOST'S DEFENSES

Natural Immunization

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 small pox that the whole science of immunology sprang.

Most virologists have accepted the fact that all immunity to viruses is mediated through antibody, and that the effectiveness of the protection depends first on the amount and the character of the antibody to protect the particular cells that are exposed to infection. 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 kills in the blood a few days after infection and reaches a peak in two or three weeks. The body continues to produce antibodies 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 sysmptoms. 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 plass through the blood, the large amounts of antibodies there waylay any virus in a second invasion and kill it 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 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 circulate through the blood stream. (Burnett, 1951).

Artificial Immunization

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 virus to be so virulent that every human cell could be invaded and every human being killed in some ghastly epidemic. Having murdered its host, the virus itself would perish just as completely. This tolerable equilibrium between man and a common virus can be explained as follows: the first contact with the virus normally takes place in childhood. 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. (Burnett, 1951).

It is against virus diseases not commonly present in their own communities that people most need the protection of articicial immunization; immunity being 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. However, in many cases it is not possible to produce a variant that is both effective and safe. Then, the only alternative is to inject relatively large amounts of killed virus, which is on the whole, not a particularly satisfactory method. (Burnett, 1951).

TISSUE CULTURE AIDS THE STUDY OF POLIO

When John F. Enders and his co-workers in 1949, originated the tissue culture method for cultivating viruses, they not only refuted earlier misconceptions of the *in vitro* propagability of viruses but they made known the potential usefulness of the cultivated cell as a test host. By 1952 the mass-produced cell culture was a reality in the laboratory; its development was accelerated by the use of antibiotics for contamination control, but the growth of cells by monolayer on stationary glass, by the revival of trypsinization for dispersal of cells, and by the simplification of the dispersing apparatus of Evans. (Syverton, 1957).

Tissue culture is the method by which the tiny cells of which all living creatures - plants and animals - are made up, are taken out of the body, grown under controlled conditions, and studied as living functioning individuals so that we may better understand how they interact in the body to bring about growth and to produce those states which we call "health" or "disease". (Melnick, 1952).

Until nearly the end of the nineteenth century, poliomyelitis was known only as a sporadic and relatively rare disease. During the era of Pasteur and Koch it began to assume epidemic status. Since then polio has risen and flourished while other infectious diseases, one by one, were broughtunder control. Now, a check in its career has occurred, not by the vagaries of nature that govern epidemics, but by conscious human intervention. (Syverton, 1957).

The discovery of a way to grow poliomyelitis virus in a tissue culture - made by John F. Enders, Thomas H. Weller and Frederick C. Robbins at the Children's Hospital in Boston for which they received the

Nobel Prize in Medicine - gave tremendous impetus to the study of this disease. It meant the end of the "monkey era" in polio research and opened the way to a much wider attack on the problem. Now that experimental work in polio is not longer dependent mainly on the infection of monkeys, many more investigators are working on the problem and new kinds of experiments are undertaken.

The chief obstacle to experimental investigation of the disease had been that it attacks only man and the other primates (although rare strains of the virus can infect certain rodents). (Melnick, 1952).

Using monkeys for detecting the presence of the virus in tissue culture and to grow it for laboratory uses posed many problems. The monkeys had to be trapped in Asia or Africa and transported to this country; a polio laboratory had to be equipped with special facilities to care for the monkeys and the monkeys nutritional needs and susceptibility to diseases had to be reckoned with. At best the monkey assay method was cumbersome and slow.

The tissue culture method has changed things. The monkeys have been replaced by test tubes in which the virus is grown in cultures of tissues. We now have a relatively simple and rapid test for detecting the presence of polio or its antibodies. (Melnick, 1952).

The materials used in the new culture technique are certain monkey or human tissues, the latter being available after some types of surgical operations. They are cut into tiny fragments about 1/25 inch square. When such a piece is bathed in a suitable nutrient medium, long strands of cells grow out from it within a few days and continue to grow for several weeks. If virus is added to the test tube, the strands of cells are broken up and destroyed, usually within a few days. That these destructive changes are specifically associated with the growth of virus is shown in two ways. The first is that they fail to occur in uninoculated tubes. The second is that the addition to the culture tube of serum containing specific antibodies for polio virus prevents their destruction of cells. This method is useful for the classification of polio viruses and for the quantitative measurement of antibodies to each type. (Melnick, 1952).

It has been established that more than one species or type of polio virus exists in nature. Each can cause the human disease. It has been found in monkeys that infection with one type confers little or no immunity against the others. The clinical disease is separable into three types, the abortive, the non-paralytic, and the paralytic. This may explain why paralytic polio is sometimes contracted more than one time by the same person.

Tissue culture methods have provided virologists with a simple <u>in vitro</u> method for testing a multitude of chemical and antibiotic agents for their effect on the multiplication of viruses in living cells. Some workers in the poliomyelitis field have already found that certain antimetabolites suppress the growth of the virus in tissue culture. These organic chemicals are structural analogues of compounds found within normal cells, and for this reason they interfer with the normal pathways of metabolism. It is thought that if these pathways can be temporarily blocked, the parasitic virus within the cell may find itself in an environment unfavorable for its propagation. (Melnick, 1952).

Humanity owes a debt of thanks to Dr. Enders, Weller and Robbins for their tissue culture technique. The day, when poliomyelitis virus will be brought under control seems much closer now due to their invaluable contribution to society.

SUMMARY

Research contributes invaluable knowledge to society and over a period of time yields considerable returns for a relatively small investment. Basic research would lose its potency in adding significantly to knowledge and understanding of nature, if it were circumscribed by the requirement that it justify its cost to the public by proving its value through immediate practical benefits. The most to be expected is to show, as I have, how certain great contributions to health and other public benefits were dependent upon continuous research.

Research is so closely identified with cultural as well as technological progress that this alone provides sufficient reason for supporting it.

Many new compounds of remarkable organic structure and biological function continue to emerge from the large scale research laboratories. One of the main activities for several years has been the screening of thousands of cultures isolated from soils of the worlds in order to find newer and better antibiotics. The discovery of penicillin by Sir Alexander Fleming was indeed a beneficial accident. There are numerous used for antibiotics; these include everything from sprays for the arrest of bacterial and fungus diseases of plants to agents for increasing fecundity in cattle, agents for short-term preservation of foods and agents for combatting human diseases.

The science of Immunity sprang from Jenner's early vaccination attempts against small pox. Most virologists today tend to agree that all immunity to certain diseases is mediated through antibodies. Through research, various theories were advanced concerning antibody formation. These date from Ehrlich and have been revived by Jerue, Talmadge and Burnet.

The bacteriophage has enhanced the study of viruses. To the early scientists the "phages" were curiosities but through them we learned all that we now know about plant and animal viruses. Through research we have learned how the virus reproduces, what is is composed of and also how it attacks the cells.

Nutritional studies led to the development of the tissue culture by Dr. John Enders and his co-workers. This, in turn, led to the growth of viruses outside of the body which led to the development of the polio vaccine. All of these many discoveries were made possible through continuous basic research.

BIBLIOGRAPHY

Burnet, F. M., "Viruses." Scientific American, XVIII (1951), 43-51

Burnet, F. M., and Frank Fenner, The Production of Antibodies. (London, 1953), p. 1.

Burkhalter, Paul, "Antibiotics." Science, CXXIX, May, 1959, p. 1457-1465.

Day, Robert, "Where Is Science Taking Us." Saturday Review, XLII, February 7, 1959, p. 49.

Demerec, M., "Origin of Bacterial Resistance to Antibodies." Jour. Bact. LVI, January, 1948, p. 63-74.

d'Herelle, F., The Bacteriophage and Its Behavior. (Baltimore, 1926), p. 629.

Gray, George, "Pauling and Beadle, " Scientific American, CLXXX, May 1949, p. 20-22.

Hutchings, Edward, Frontiers in Science, (New York, 1959), p. 3-8.

"Immunity," Encyclopedia Britannica, (Chicago, 1961). XII, 112.

Irving, George, and Horace Herrick, Antibiotics. (Brooklyn, 1949), p. 4-369.

Keefer, C. S. and F. G. Blake, "Penicillin in The Treatment of Infections." Journal of American Medical Association, CXXII, (1943), p. 1217.

Lessing, Lawrence, "The National Science Foundation Takes Stock," Scientific American, CLXXXIX, March, 1954, p. 33.

Melnick, Joseph, "A New Era In Polio Research," Scientific American, CLXXXVII, November, 1952, p. 27-29.

Pelczar, M. J., and Roger Reed, Microbiology, (New York, 1958), p. 270-275.

"Penicillin," Encyclopedia Britannica, (Chicago, 1961), XVII, 464.

Pollard, Ernest, "The Physics of Viruses," Scientific American, CXCI, December, 1954, p. 64.

Robinson, F. A., Antibiotics, (New York, 1953), P. 23

Rodriques, L. D., Bacteriophage, (Noticias Form, 1945), 441-427.

"Science The Citizen," Scientific American, CXCVI, May 1957, p. 63

Selye, Hans, "What Makes Basic Research Basic?" Saturday Evening Post, CCXXXI, January 24, 1959, p. 30-34.

Sheehan, J. C. and K. R. Henery-Logan, "Synthetic Penicillin," Science, CXXV, March, 1957, p. 591.

Sokoloff, Boris, Unconquered Enemy (New York, 1941), O. 129.

Stent, Gunther, "The Reproduction of Bacterial Viruses," Scientific American, CLXXI, May, 1953, p. 36.

Syverton, Jerome, "Comparative Studies of Normal and Malignant Human Cells In Continuous Culture," New York Academy of Sciences, V, March, 1957, p. 333.

Waterman, Alan, "Basic Research," Bulletin of Atomic Scientists, XIII, December, 1957, p. 369.

Weaver, Warren, "The Encouragement of Science," Scientific American, CXCIX, September, 1957, 170-178.

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