

That confidence and that faith are  
the essence of Medical Research & the  
spirit of the Laboratory which is the production  
Centre whence issue those fundamental  
principles that, often unexpectedly,  
revolutionize medical thought and change  
medical practice —

To the Directorate of the Passano  
Foundation, to Mrs Passano Sr., and to all of  
you who honor me by your presence  
this evening I express my deep sense  
of appreciation & my warmest thanks

Avery, Oswald Theodore, 1877-1955.  
Papers, 1879-1970  
5 speeches.

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Dr Eastman, Members of the Board of Directors & Staff  
of Passano Foundation.

MS. DIV. 70-128  
AC. NO.

I esteem it a signal honor to have been chosen as  
the 1949 recipient of the distinguished Passano Award  
Dr Eastman, Dr Irons and President Gill have been  
far too gracious & generous in their appraisal of the  
my share in the work for which this Award is granted  
me. Whatever merit may accrue from these studies  
investigations is due in part to the happy auspices  
under which the work has carried on, and in large  
measure to the devoted cooperation of my associates  
without whose aid & collaboration I would not  
be honored as I am this evening in receiving  
this high distinction.

<sup>This Award</sup>  
I am the more happy that it pays  
tribute to the vision, ideals & character of the  
man whose name it bears. ~~and~~ that it is an  
earnest of the confidence of his colleagues  
that Truth is discoverable and of their faith that  
Truth is worth discovering.

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Then confidence & faith are the essence  
of the entire research, and the spirit of  
the great pre-eminence,  
Lovingly, this is some wise fundamental  
principles, often unexpectedly, revealing  
more than the most the change in  
reading

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MAN AND MICROBES

ACQ. NO. 10-128

As the subject of my remarks this evening I have borrowed the title of a book called Man and Microbes, written by Dr. Stanhope Bayne-Jones and published in 1932 by Williams and Wilkins as one in a series in connection with the celebration of the Century of Progress Exposition. This book admirably describes the thrilling story of our knowledge of microbiology and the ways in which this knowledge has influenced the life of man and changed the courses of civilization.

Microbes are everywhere about us; in the air, in the soil and in the waters that course through and surround the earth, there dwell myriads of microorganisms of diverse kinds invisible to the unaided eye of man. Happily, the vast majority of them are beneficent in their action, indeed indispensable to the life of higher plants, animals and man. For example, microorganisms fulfill useful and important functions in agriculture and in maintaining the fertility of the soil. Working together in cooperation, each according to its kind and abilities they perform chemical tasks essential in the economy of nature; decomposing giant molecules of protein, fermenting complex carbohydrates and splitting fats into simpler forms they salvage from dead organic matter and return into circulation those elemental substances upon which depend the continuity and renewal of life. Indeed, as Bayne-Jones has pointed out, microorganisms are the original chemical pioneers of the world. Far from being the simplest and most primitive form of life, they were originally thought to be, they are now known to possess structural complexity and biochemical organization significant of the operation of common principles everywhere in animate nature and indicative of the chemical unity of life from microbes to man himself.

With increasing knowledge of the biochemical activities of microorganisms, man now uses them as specific agents for the carrying out of

important agricultural and industrial processes in the accomplishment of which they exceed the competence and technical skill of the most expert biochemist. The harnessing of microbes in the service of industry was forecast by Pasteur when he said, "A day will come, I am convinced, when microorganisms will be utilized in industrial processes on account of their ability to attack organic matter". That prophesy has long since been fulfilled. Today microorganisms are used as biochemical tools in solving intricate problems in human physiology and in comparative biochemistry. They are employed as selective agents in the bioassay of vitamins and amino acids. The isolation from soil bacilli of an enzyme capable of attacking creatinine has provided biological methods for the study of protein metabolism. And much of the knowledge of the intermediate metabolism of carbohydrate and its significance in the understanding of muscle physiology received its initial impetus from the recognition of the nature of alcoholic fermentation by yeast. If time permitted, one might go on citing instance after instance of the manifold ways in which microorganisms have contributed to human welfare and rewarded man in his search for truth.

Although it is evident that microbes are the loyal allies of man, it is also true that certain other varieties are his ruthless foes. Just as in the universe in which we live, not all human beings are men of good will, so in the microbial world there exists a willfull minority of microorganisms that are harmful and destructive. Some of these are antisocial and antagonistic to the growth and life of their fellow microbes, while others are the causitive agents of highly infectious diseases of man.

The phenomenon of bacterial antagonism is not new. It has long been known that when two different species of microorganisms are grown together, one of them may elaborate and secrete into the medium of their environment a substance which is antagonistic and often fatal to the life of the other

species. In 1877 Pasteur observed in the course of his studies on anthrax that "When a culture of the anthrax bacillus is seeded at the same time with a common air microorganism, the anthrax bacilli grow only poorly and sooner or later die. It is a remarkable thing, said he, that the same phenomenon is seen in the body even of those animals most susceptible to anthrax, leading to the astonishing result that anthrax bacilli can be introduced in profusion into an animal which does yet not develop the disease"... He further stated, "These results perhaps justify the highest hopes for therapeutics." But it is only in recent years with further advances in knowledge of the biochemical activities of microorganisms that this principle has been practically applied in the therapy of human infections. From soil bacillus Dubos isolated the antimicrobial substances, Gramicidin and Thyrothridin; from a common bread mold Fleming discovered Penicillin; from another species Waksman derived Streptomycin. These are among the most beneficent gifts of microbiological science to the welfare of mankind.

Now let us turn to the group of microorganisms known as the pathogenic bacteria. Among these I have chosen to speak of the pneumococci, not because I regard them as the most important or that our knowledge of them is more complete, but rather because they happen to be the group with which I am most familiar and the study of which has revealed certain facts which serve to illustrate the interdependence of medicine and the other sciences of nature.

You are all familiar with the fact that the large majority of all cases of bacterial pneumonia are caused by the pneumococcus. Although all pneumococci grow alike in artificial media, and closely resemble each other under the microscope they have, nevertheless, been classified into a number of sharply defined and specific types. These type differences are not detectable by the ordinary methods of bacteriology, but require for

their demonstration the more subtle methods of serology and immunology. The biological classification, based on these methods, was worked out and clinically applied before there was any knowledge of the chemical nature of the substance upon which type specificity is now known to depend. The recognition of the existence of pneumococcal types made it possible to determine the relative frequency of their occurrence in pneumonia and to recognize differences in the severity and mortality of the infections they produce. A study of the distribution of the various types in health and disease has given a new interpretation of the epidemiology of pneumonia; provided biological methods for tracing the origin and spread of infection; the mode of dissemination of the disease producing types in the mouth secretions of convalescent and normal individuals, and the detection of the carrier state in patients and healthy persons. The knowledge of the classification and the principles involved revealed the importance of type-specific antibodies in the mechanism of recovery from pneumonia and lead to the development and clinical use of anti-pneumococcal serum, the only effective and specific form of treatment prior to the advent of the sulfonamides and antibiotics.

In the course of these studies, the question naturally arose, why should pneumococci, which are otherwise so similar, exhibit such diverse and sharp differences in their serological reactions? What is the chemical basis of their biological specificity, and what constituent or structure of the bacterial cell is responsible for these differences in behavior? The answer to these questions lies in the following facts:

During growth in an optimal medium and especially when growing in the blood and tissues of a susceptible host, pneumococci elaborate a viscous, gum-like material which surrounds the cell body, forming a structure known as the cell capsule. This capsular substance diffuses into medium of their environment and in soluble form retains the type specificity of the organisms

from which it is derived. This soluble specific substance is found, not only in the cell-free, cultured filtrates, but also in the blood and urine of patients during the course of pneumococcal pneumonia. The capsular substance has been isolated from various types of pneumococcus and in all instances has proved to be a complex carbohydrate, a polysaccharide. But interestingly enough, the capsular polysaccharide from each specific type is chemically different, possessing unique properties that serve to distinguish it from that of all other types. Indeed, the capsular polysaccharides are as chemically distinct as they are serologically specific for each type. Thus, type specificity is determined by the chemical individuality of the particular polysaccharide present in the cell capsule. This fact is all the more striking since hitherto only proteins were thought to participate in immunity reactions.

While there is no proof that the capsular polysaccharides are primarily toxic, there is evidence that they can interfere with the natural processes of recovery from pneumococcal pneumonia. Because of the avidity with which they combine with antibodies in the patient's blood they tend to neutralize these protective agents before they can reach the focus of infection. Moreover, the capsular polysaccharides are known to inhibit phagocytosis, an important protective mechanism of the body whereby the white blood cells seek to remove and destroy the invading bacteria.

Like other bacteria, pneumococci, growing in an unfavorable environment, can undergo variations, resulting in changes in cellular structure and function. Under these conditions, fully encapsulated and virulent cells give rise to variants which have lost the capacity to form capsules, and with the loss of capsules the cells also lose their type specificity and virulence. The variants are referred to as the rough or R forms, indicating merely that the surface of the colonies they produce on solid media are rough in contrast to the smooth colonies of the encapsulated cells which

are called smooth or S forms. The change from encapsulated S forms to non-encapsulated R variants is often reversible. In many instances these variants can be caused to regain their capsules by animal passage or by serial transfers in a medium containing anti - R serum. When reversion occurs, the variant R cells always revert back to the same specific type as that of the encapsulated parent culture from which they originally arose. This reversible change within a single type is quite different from that which involves the actual transformation of one specific type into another and wholly different specific type. The phenomenon of transformation of pneumococcal types has never been observed to occur spontaneously but can be experimentally produced at will in predetermined ways depending upon the type specificity of the substance used to induce the reaction. The phenomenon was first discovered by Griffith in 1928. A single example will illustrate the method he originally used and serve to indicate the variety of transformations possible among the known types. For instance, Griffith observed that when a small number of living, unencapsulated R variants derived from Type II are added to a suspension of heat killed, encapsulated Type III cells and this mixture is injected into mice, many of the animals so treated died. From the blood of the mice that succumbed he recovered living, encapsulated Type III pneumococci in pure culture. This surprising result is all the more startling since the Type III organisms originally injected were dead cells and the living R culture used was wholly incapable, by itself, of causing fatal infection. These experiments were adequately controlled and the results have since been confirmed by other investigators. These experiments were originally done in mice, and all attempts to induce the reaction without the intervention of the animal were unsuccessful. Subsequently, Dawson and Sia succeeded in inducing transformation In vitro. This they accomplished by growing the variant (R) strain in a fluid medium

containing anti - R serum and killed encapsulated (S) cells. Thus they showed that transformation can be induced in the test tube as well as in the animal body. Later, Alloway prepared active extracts of encapsulated pneumococci from which all formed elements and cellular fragments had been removed by Berkefeldt filtration. Active extracts containing the transforming agent in soluble form are as effective as are the intact cells from which they were prepared.

Pneumococcal extracts are crude mixtures of complex substances comprising the proteins, polysaccharides, lipids and other compounds of which the bacterial cell is composed. I will not burden you with details of the technical procedures and chemical methods used in attempts to isolate the substance upon which the biological activity of the extracts is now known to depend. It will suffice merely to mention that by chemical fractionation, by the use of specific enzymes and serological reactions, it was possible to eliminate inert and irrelevant material and finally to isolate, in highly purified form, a substance possessing the specific transforming activity of the original extracts.

The data obtained by chemical analysis of the active substance, together with determinations of its physical properties by electrophoresis, ultracentrifugation and ultraviolet spectroscopy indicate that it contains no demonstrable protein, lipid or serologically reactive polysaccharide, and that it consists chiefly, if not exclusively, of a nucleic acid of the desoxyribose type. Quantative titrations of the biological activity of the substance isolated from Type III cells have shown that an amount as small as 0.003 microgram, representing a final dilution in the reacting system of 1 part in 600 million, is capable of inducing specific transformation of R cells into Type III pneumococci.

Further studies have revealed certain facts which contribute to the understanding of the mechanism of the transformation reaction. For example,

it is known that not all non-encapsulated R cells are responsive to the stimulus of the transforming substance; some fail to respond while others react to minimal amounts. These differences in the physiological response of R variants are reflected in corresponding difference in certain characteristics of the colonies they produce. This fact makes it possible to isolate "competent" or R strains in pure culture. However, even these cultures can undergo spontaneous dissociation, giving rise to other variants which are wholly incompetent and incapable of reacting with the transforming substance.

Furthermore, it was early discovered that the addition of blood serum or serous fluids to the nutrient medium used in the test is essential, since in its absence transformation does not occur. It is evident then, that serum provides certain accessory factors that play an important part in the interaction of competent R cells and the active transforming principle. Two components of serum have been identified, neither of which is active by itself, but together they function as well as does the whole serum. One of these serum components is the R antibody protein that agglutinates the growing R organisms, forming large aggregates of agglutinated cells which fall to the bottom of the culture tube where they create, presumably due to their reducing action, environmental conditions necessary for transformation. The other component is the serum albumin which, in its native state or in crystalline form, appears to prepare the cells for the ready uptake of the transforming substance.

On the basis of the experimental evidence, it appears that both the physiological state of the R cells and the environmental factors I have just cited determine the biochemical events leading to the interaction between the living bacteria and the transforming substance. Once this has taken place the ultimate outcome suggests that the active principle intervenes in the metabolism of the R cells, giving rise to a series of

coordinated enzymatic reactions which culminate in the synthesis of a new capsular structure.

Whatever the mechanism may be, the experimental findings clearly demonstrate that the induced alterations in cellular structure and function are not random changes, but are predictable always corresponding in type specificity to that of the encapsulated cells from which the transforming substance was isolated. Once transformation has occurred, the newly-acquired characteristics are thereafter transmitted in series through innumerable transfers in artificial media without any further addition of the transforming agent. And from the transformed cells themselves, a substance of identical activity can again be recovered in amounts far in excess of that initially used to induce the transformation. These induced changes are not temporary modifications but permanent alterations that persist, provided the cultural conditions are favorable for the continuance of capsular development. It is evident that the inducing substance, which evokes the synthesis and determines the specificity of the capsular polysaccharide, is itself reduplicated in the daughter cells. It is also significant that the substance inducing the reaction and in turn the substance produced in response to it are chemically distinct, each belonging to a wholly different class of chemical compounds.

The accumulated evidence justifies the conclusion that the fundamental chemical unit of the transforming substance is a nucleic acid of the desoxyribose type. In view of this, nucleic acids of this type must now be regarded, not merely as structurally important, but as functional active substances determining the biochemical activities and biologically specific characteristics of the cells of which they form a part.

In closing, I take pleasure in acknowledging, on this occasion, my gratitude and thanks to those colleagues of mine whose expert knowledge and loyal support has made possible the work in which I have been

privileged to share. To them I am indebted for an association which has meant much to me personally, and has contributed greatly to the research problems I have briefly reviewed this evening. And may I again express to the Directors of the Foundation my deep sense of appreciation of being enrolled as a recipient of the distinguished Passano Award.

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