The "Undiscovered" Discovery

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Prologue

THIS STORY deals with the very important discovery that DNA could possess and transmit genetic information and some aspects of the scientific climate existing at the time which probably were responsible for the fact that the discovery was really "undiscovered," generally unappreciated, and failed, for several years, to affect the trend of science. It is written in honor of Thomas Francis, Jr., MD, because, when Dr. Francis came to the hospital of the Rockefeller Institute for Medical Research in the summer of 1928 to do some research under the supervision of Oswald T. Avery, MD, he became interested in bacterial transformation. He tried to obtain the transformation of a type of pneumococcus in vitro but unfortunately did not succeed. The time was not yet ripe, for later when Alloway came to work with Dr. Francis, he took on the problem and did succeed as did Dawson and Sia later on. Dr. Francis never returned to research on bacterial transformation, but during the time he was at the Rockefeller Institute he developed a close and lasting friendship with Dr. Avery, the main architect of the DNA discovery. They shared an active interest in respiratory disease, Dr. Francis mainly with influenza, and Dr. Avery, with pneumonia, and they enjoyed getting together for late afternoon social hours during which science was often discussed. In this one respect it is unfortunate that Dr. Francis left the institute to go to New York University in 1938 and then on to the University of Michigan in 1941, for I know he would have greatly enjoyed a continuation of his close association and almost daily conversations with Dr. Avery during the exciting years immediately preceding the publication about transforming DNA by Avery, MacLeod, and McCarty in 1944. Dr. Francis had a great capacity for friendship.

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The Discovery

The first of a class of substances to be called "nucleic acids" by Altman in 1889 was isolated by Miescher in 1869. As so often happens with a discovery, Miescher was somewhat uncertain about the nature of the nonprotein, phosphorus-containing material he had obtained from pus cells. He called it a "nuclein" and described its properties in a paper he submitted to Hoppe-Seyler who was so perplexed he held up publication while he and his students repeated and extended Miescher's experiments. Hoppe-Seyler became satisfied that Miescher's results were correct, for in his journal in 1871 appeared not only Miescher's paper but three from Hoppe-Seyler's laboratory plus an additional one from Miescher, all on this new class of substances. Miescher extended his work to include fish sperm, and, in 1879, Kossel, working in Hoppe-Seyler's laboratory, entered the field and gradually gained control. Kossel dominated the field until the entrance of Levene about the turn of the century.

The structural organic chemistry of the nucleic acids was worked out in beautiful detail by Levene and co-workers during the next 35 years. Levene considered DNA to be a tetranucleotide containing repeating units of the bases adenine, guanine, cytosine, and thymine. In the book *Nucleic Acids* by Levene and Bass published in 1931 they state on p 262:

On the basis of the data discussed in previous chapters, namely, on the basis of the isolation of four bases in equimolecular proportions on complete hydrolysis of ribodesose nucleic acid and on the basis of the isolation of four nucleosides on partial hydrolysis, it is warranted to attribute to the substance a tetranucleotide structure.

On pp 276 to 277, it is stated:

Thus the tetranucleotide structure of yeast nucleic acid has been re-established by the analytical method. It will be seen later that the formulation of Levene has been confirmed by the physicochemical method.

Even at that time Levene was uncertain about the molecular weight, for on p 289 the following occurs:

On the other hand, it must be borne in mind that the true molecular weight of nucleic acids is as yet not known. The tetranucleotide theory is the minimum molecular weight and the nucleic acid may as well be a multiple of it.

Probably because of the general acceptance of the tetranucleotide theory no attempt seems to have been made to relate biological activity to the nucleic acids. The early biologists certainly connected the nucleus of the cell to biological activity, but because of the diversity displayed by protein, tended to believe that protein played the central role in the transmission of genetic properties. One of the first clear statements was that of the great American cytologist Edmund B. Wilson who in 1895 stated,

Chromatin is known to be closely similar to, if not identical with, a substance known as nuclein, which analysis shows to be a tolerably definite chemical compound composed of nucleic acid and albumin. And thus we reach the remarkable conclusion that inheritance may, perhaps, be effected by the physical transmission of a particular chemical compound from parent to offspring.

The effect of the Levene school of thought is clearly evident for in 1925 Wilson wrote in his book *The Cell* the following:

Apart from the characteristic differences between animals and plants, the nucleic acids of the nucleus are on the whole remarkably uniform, showing with present methods of analysis no differences in any degree commensurate with those from the various species of cells from which they are derived. In this respect they show a remarkable contrast to the proteins, which, whether simple or compound, seem to be of inexhaustible variety. It has been suggested accordingly, that the differences between different "Chromatins" depend upon their basic or protein components and *not* upon their nucleic acids.

There is little wonder that scientists in the 1930's considered proteins to be important in genetic transmission and showed little interest in the nucleic acids, apart, of course, from their structural organic chemistry. A further indication of this lack of interest is the fact that in their 900-page volume *Practical Physiological Chemistry* published in 1931, Hawk and Bergheim devoted only 12 pages to nucleic acids and nucleoproteins. The discussion indicates that the nucleic acids could be divided into two classes—animal and plant—with the former containing deoxypentose rather than pentose and thymine rather than uracil. Levene was credited with the structural work supporting the tetranucleotide theory. I am convinced that the general acceptance of this theory was largely responsible for the reluctance on the part of scientists to consider that nucleic acids might carry biological and especially genetic information. This was, therefore, the scientific atmosphere surrounding the early work on the transforming principle of the pneumococcus. When Avery, who had been working on and off on transformation since 1928, finally directed the work toward the purification and isolation of the agent responsible for bacterial transformation, he certainly did not expect to end up with nucleic acid. As the experimental work conducted with his two younger associates, MacLeod and McCarty, proceeded it became obvious that the transforming principle was in fact DNA. Finally a paper was submitted on Nov 1, 1943, and published Feb 1, 1944, in the *Journal of Experimental Medicine* entitled "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types: Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated From Pneumococcus Type III."

One has only to read this paper in detail to recognize the turmoil and mental anguish these investigators must have gone through as the work proceeded. Although mentioned in the title, nucleic acid is not mentioned in the first eight pages of the paper. On p 9, a table showing the results of elementary chemical analysis is presented with the statement that while there was close agreement with the figures for nucleic acid, the analytical figures by themselves did not establish that the isolated substance was a pure entity. Obviously, although the results indicated otherwise, they were still thinking about the possibility of a trace of highly biologically active protein. They tested their preparations extensively with known enzymes and tissue extracts since certain of these were found to destroy the biological activity. They found an enzyme that would destroy the activity. This was the enzyme reported from Greenstein's laboratory in 1940 and called "deoxyribodepolymerase" by Greenstein in 1943.

Finally after extensive enzymatic and serological analysis and some physicochemical and quantitative studies, the tentative conclusion was reached on the 14th page of the paper that:

> The fact that transforming activity is destroyed only by those preparations containing depolymerase for desoxyribonucleic acid and the further fact that in both instances the enzymes concerned are inactivated at the same temperature and inhibited by fluoride provide additional evidence for the belief that the active principle is a nucleic acid of the desoxyribose type. . . .
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> Thus in both the electrical and centrifugal fields, the behavior of the purified substance is consistent with the concept that biological activity is a property of the highly polymerized nucleic acid. Ultraviolet absorption curves showed maxima in the region of 2600 A and minima in the region of 2350 A. These findings are characteristic of nucleic acids.

Yet in the immediately following paragraphs the terminology that was used was "transforming principle" or "active material," and not "nucleic acid." The paper contains an excellent discussion and the summary is as follows:

> 1. From Type III pneumococci a biologically active fraction has been isolated in highly purified form which in exceedingly minute amounts is capable under appropriate cultural conditions of inducing the transformation of unencapsulated R variants of Pneumococcus Type II into fully encapsulated cells of the same specific type as that of the heat-killed microorganisms from which the inducing material was recovered.
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> 2. Methods for the isolation and purification of the active transforming material are described.
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> 3. The data obtained by chemical, enzymatic, and serological analyses together with the results of preliminary studies by electrophoresis, ultracentrifugation, and ultraviolet spectroscopy indicate that, within the limits of the methods, the active fraction contains no demonstrable protein, unbound lipid, or serologically reactive polysaccharide and consists principally, if not solely, of a highly polymerized, viscous form of desoxyribonucleic acid.
>
> 4. Evidence is presented that the chemically induced alterations in cellular structure and function are predictable, type-specific, and transmissible in series. The various hypotheses that have been advanced concerning the nature of these changes are reviewed.

The final conclusion in the paper is simply and clearly stated but in a manner characteristic of a very conservative scientist as follows: "The evidence presented supports the belief that a nucleic acid of the desoxyribose type is the fundamental unit of the transforming principle of Pneumococcus Type III."

Clearly the evidence presented was substantial and the investigators recognized that they had made a significant discovery. Why, therefore, was this great discovery not immediately recognized by the scientific world and why did it not influence the direction of biomedical research? Why did not the discovery that nucleic acid could carry and transmit genetic information receive the recognition that it so richly deserved, for this was a major discovery, one contrary to general thought, and hence one that should have immediately affected scientific thinking in several fields. I am convinced that an unfortunate combination of circumstances was responsible. Perhaps of major importance was the fact that the discovery was quite contrary to the dominant thinking of many years and, hence, required not only a vigorous presentation but also a vigorous and continuing promotion for acceptance. This was not forthcoming. In fact, although the authors made the correct conclusion based on the scientific evidence, they were modest and somewhat cautious in their presentation. In their paper, after the first statement of their "belief that the active principle is a nucleic acid," they returned in the very next and succeeding paragraphs to terms such as "active material" and "transforming substance." In the discussion they stated:

> So far as the writers are aware, however, a nucleic acid of the desoxyribose type has not heretofore been recovered from pneumococci nor has specific transformation been experimentally induced *in vitro* by a chemically defined substance,

thus expressing surprise not only in finding DNA in pneumococci but in the biological activity. Later on they said:

> If it is ultimately proved beyond a reasonable doubt that the transforming activity of the material described is actually an inherent property of the nucleic acid, one must still account on a chemical basis for the biological specificity of its action.

Similar hedges about the possibility of impurities and about the possibility of confirmation of the results are in the summary. Thus it would appear that they felt that they had not proved the point "beyond a reasonable doubt," and thus did not tend to imbue the reader with confidence in their results. Yet today most scientists would regard their evidence as impressive.

Another factor was Dr. Avery himself. At the time of the writing of the paper he was 66 years of age and planning his retirement. He was a warm and wonderful man of great intelligence but nevertheless a cautious and timid man. He had spent most of his scientific effort on the pneumococcus and, although he had made many important contributions, his dream of conquering the dreaded acute lobar pneumonia by means of specific sera had just been punctured by the discovery and development of the sulfa drugs. He had encouraged work on the transforming principle over many years. Perhaps the best picture of Dr. Avery at this time can be gained from a letter he wrote to his brother, Roy C. Avery, on May 13, 1943. This letter should be published in full sometime but some excerpts may suffice here. After relating the ups and downs of the early work on the transforming principle, Avery comments, "Some job—full of heartaches and heartbreaks. But at last, *perhaps* we have it." After describing some of the properties of the nucleic acid Dr. Avery concluded, "This does not leave much room for impurities—but the evidence is not great enough yet." Later on he stated, "If we are right—of course that's not yet proven." Near the end of the long letter he wrote:

> Sounds like a virus—may be a gene. But with the mechanisms I am not now concerned —one step at a time and the first step is, what is the chemical nature of the transforming principle? Someone else can work out the rest. Of course, the problem breathes with implications. It touches the biochemistry of thymus type of nucleic acids which are known to contribute the major part of chromosomes but have been thought to be alike regardless of major species. It touches genetics, enzyme chemistry, cell metabolism, and carbohydrate synthesis, etc. But today it takes a lot of well documented evidence to convince anyone that the sodium salt of desoxyribose nucleic acid, protein free, could possibly be endowed with such biologically active specific properties and this evidence we are now trying to get. It's lots of fun to blow bubbles but it's wiser to prick them yourself before someone else tries to. So there's the story Roy—right or wrong its been good fun and lots of work. This supplemented

by war work and general supervision of other important problems in the lab has kept me busy, as you can well understand. Talk it over with Goodpasture but don't shout it around—until we're quite sure or at least as sure as present methods permit. It's hazardous to go off half-cocked and embarrassing to have to retract later. I'm so tired and sleepy I'm afraid I have not made this very clear—but I want you to know—and sure you will see that I cannot well leave this problem until we've got convincing evidence. Then I look forward and hope we may all be together—God and the war permitting and live out our days in peace.

It would appear that by the time the paper was submitted for publication the following November, Dr. Avery, despite the terminology used in the paper, had convinced himself that the work and the conclusion were correct and he was ready to retire, leave the rest to others and to live in peace. The war was still on, making especial demands of the younger men. The interests of his two younger physician associates had changed, for MacLeod had left to accept a position as professor at New York University School of Medicine in 1941 and McCarty had accepted additional obligations by joining the Rockefeller US Naval Medical Research Unit in 1942. Although two additional papers on the transforming substance were published in 1946 by McCarty and Avery, one on the effect of deoxyribonuclease and the other on an improved method for isolation, Avery had no desire to argue the merits of the discovery before the scientific world at that time, a world that was fully preoccupied with the war. I am sure that he felt the pride of accomplishment within himself and that sooner or later the world would recognize that accomplishment. But the fact remains that no one undertook the task of describing the discovery and arguing its merits and significance before scientific audiences across the nation; hence, several years passed before there was general acceptance.

The Rockefeller Institute for Medical Research, as an organization, seems to have given scant official attention to the discovery. In the 1944-1946 "Descriptive Pamphlet" of the institute there is the statement:

> More recently it has been found that desoxyribonucleic acid is intimately associated with the structural organization of pneumococci and, indeed, that certain nucleic acid polymers of the desoxyribose type possess the capacity, under appropriate conditions, to induce transformation of the various types of pneumococci. Thus, the nature of the capsular polysaccharide appears to be dependent upon a metabolic system which at some point is specifically oriented by desoxyribonucleic acid.

The identical words appear in the 1946-1948 "Descriptive Pamphlet," and there is no evaluation whatsoever of the significance or importance of the discovery. One would never guess that one of the really great discoveries within the history of the institute was being discussed. Hotchkiss, then at the institute, did take up research on the transforming DNA and made important contributions as did Alexander, Leidy, and Zamenhof.

Still another factor that undoubtedly contributed to the overlooking of the work on the transforming principle was the series of important scientific accomplishments that took place in the early 1950's. By this time Chargaff had demonstrated quite convincingly that nucleic acid could not be a simple repeat unit of the four nucleotides by showing that the base composition of different DNAs differed considerably, although A always equaled the T content and G the C content. Ideas with respect to the structure of the nucleic acids were beginning to change. In 1951, Lederberg and Zinder discovered transduction and Pauling described the α-helix for peptides and proteins. In 1952, Hershey and Chase published their famous experiment showing that the genetic information of bacterial viruses was carried in their DNA, work which shared recognition by the 1969 Nobel Prize in Medicine. Needless to say this work was generally regarded as supportive of the earlier work by Avery and colleagues and did much to secure acceptance of DNA as a transmitter of genetic information, but even then the suspicion remained strong that protein might be involved. Also in 1952 Schachman, Pardee, and Stanier discovered ribosomes and showed that they contained RNA and not DNA. In 1953, Watson and Crick revolutionized scientific thought by their presentation of the double helix for the structure of nucleic acid. And in 1956, Fraenkel-Conrat and Gierer and Schramm made the important discovery that the biological activity of the tobacco mosaic virus nucleoprotein was carried solely by the RNA of the virus, thus proving for the first time that RNA could also carry and transmit genetic information. Truly an unbelievably rich harvest of scientific discovery occurred during a five-year period, and today Lederberg, Pauling, Hershey, Watson, and Crick are Nobel Laureates as a result of accomplishments made during that period. With so much to occupy their minds and so much scientific progress to digest and assimilate, it is small wonder that scientists continued to fail to give proper recognition to the discovery of transforming DNA and simply accepted it as a fact of life. Perhaps full acceptance of the role played by nucleic acids in the storage and transmission of genetic information did not come until the genetic code and the synthesis of DNA, RNA, and proteins was understood.

These, then, are the factors which I believe to be responsible for the failure of the scientific world to accord proper recognition to the discovery of the DNA nature of the transforming principle of the pneumococcus. It is difficult to assess which, if any, of these factors was more important than the others. Certainly the scientific atmosphere at the time was very important. The general acceptance over many years of the "tetranucleotide theory" as the basis for the structure of DNA, with the great organic chemist Levene as its proponent, certainly was a major obstacle, for it was argued vigorously and convincingly that DNA could not possibly carry biological information. The work of Brachet and Caspersson in the early 1940's, relating DNA to protein synthesis within cells, had little effect. The theory died a slow and lingering death and the person most responsible for its demise, Chargaff wrote on p 368 in a book, *Nucleic Acids,* co-authored by Davidson, published in 1955:

> The tetranucleotide theory continues, for this reason, to lead a stubborn existence. There never were any but psychological reasons for its formulation, as has been pointed out before; but even in a very recent and massive treatise there will be found the statement that thymus nucleic acid is a large chain consisting of 500 to 1,000 tetranucleotide units and that each tetranucleotide is formed by the combination of four nucleotides containing adenine, cytosine, guanine, and thymine, respectively.

If the true structure of DNA had only been known and accepted, it is highly probable that the discovery by Dr. Avery and his colleagues and its significance would have received ready recognition. Furthermore, instead of a timid and unusually cautious presentation the authors might have set forth their conclusions with greater firmness and confidence and this would have fostered ready acceptance. If Dr. Avery or one of his younger associates, all of whom were trained as physicians, had been trained as a professional biochemist there might have been a difference in acceptance of the discovery, as well as a high probability that a person so trained would have wanted to carry on the work. The war was undoubtedly a major factor but one which is difficult to evaluate. Many persons felt tired and worn out by the end of the war in 1945, but had Dr. Avery been ten years younger, instead of looking forward to retirement, things might have been different. Many factors played a part and with the rush of scientific discovery of the early 1950's it is easy to understand why the discovery of transforming DNA did not receive the public recognition that it so richly deserved. But of one thing we can be sure —Dr. Avery, at the time, recognized the significance of the discovery and he was inwardly completely happy and at ease with himself. We have all come to realize the greatness of the discovery and I am sure Dr. Avery knew that this would happen, sooner or later. He did not need public acclaim for he was inwardly content because of his knowledge of the discovery and the honor accorded him by his close friends.

An Apology

In 1935, I reported the isolation of a crystallizable protein possessing the properties of tobacco mosaic virus. By 1938, as a result of discussions with Bawden and Pirie in London in July 1936 and subsequent work in their laboratory and in my laboratory, I knew this crystallizable material was a ribonucleoprotein. I also was familiar with the work that was going on in Dr. Avery's laboratory on the transforming principle and. over River's objections, I included a discussion of it in a chapter entitled "Biochemistry and Biophysics of Viruses," written for Doerr and Hallauer's *Handbuch der Virusforschung,* published in 1938. In this discussion, I commented: "This phenomenon is virus-like, and it is because of this and the fact that it may become important from the standpoint of the chemistry of viruses that a discussion is included here." After describing the method of preparation and biological properties of the purified material, I ended the discussion with:

No chemical tests were made on these purified preparations, hence nothing is known about the nature of the active agent. It is to be hoped that the study of this agent will be continued because of its virus-like nature.

I was also interested in the RNA of tobacco mosaic virus. In 1942, Cohen and I reported the isolation of this RNA with an unusually large molecular weight and we reached the conclusion "that the nucleic acid exists in thread-like molecules, the length of which is that of the intact virus molecule." It is obvious that despite my 1938 writings, I was not impressed with the significance of the 1944 discovery by Avery, MacLeod, and McCarty or I would have prepared high molecular weight tobacco mosaic virus-RNA once again and tested it for virus activity despite the fact that RNA was not suspected to have genetic properties. It remained for Fraenkel-Conrat to do this important experiment in my laboratory 14 years later. I have searched my memory and have failed to find any really extenuating circumstances for my failure to recognize the full significance of the discovery of transforming DNA. Some of the factors mentioned in the body of this paper may have had some influence, and with the outbreak of World War II in 1941 my laboratory effort was converted almost overnight to the development of preventative vaccines for our armed forces and this total effort continued until well past the end of the war in September 1945. But there should have been time for me to accord proper early recognition to the discovery of transforming DNA in 1944, and for my failure to do this I apologize. I am pleased to be able to record in this paper the fact that in 1947 Lasker Awards in Basic Research were presented to Drs. Avery and Francis.

This communication was developed from notes which were used in connection with a speech I presented on the occasion of the dedication of the Avery Memorial Gateway at the Rockefeller Institute in New York, Sept 29, 1965.