The Long Neglect of Genetic Discoveries and the Criterion of Prematurity

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In a symposium in honor of the centennial of the publication of Gregor Mendel's pioneer experiments in genetics, held at the meetings of the American Philosophical Society in Philadelphia on April 23, 1965, I delivered a paper entitled "A Century of Biochemical Genetics." After remarking on the complete lack of knowledge in Mendel's time of the biochemical nature of any hereditary material and even of the important role of the nuclei of cells in the transmission of hereditary traits, I continued with these words:

> Every historian of genetics, indeed every biologist of this century, has expressed wonder at the long neglect of Mendel's discoveries, and many reasons have been suggested. Perhaps it has not been realized as it should be that this prolonged neglect of a scientific discovery is not at all unusual; even the science of genetics abounds in such. Two of these relate, respectively, to the biochemical nature of the genetic materials and to the biochemical nature of gene action.[1]

I then recounted the history of Friedrich Miescher's discovery of the chemical basis of heredity and of Sir Archibald Garrod's discovery of no less than four "inborn errors of metabolism" in each of which a specific enzyme deficiency was identified as the cause.

In the case of Mendel, as I argued in 1953,[2] the neglect was most probably due to failure to comprehend the significance and the generality of Mendel's results, rather than the inaccessibility of his publication or the lack of interest in plant breeding at the time, reasons sometimes suggested. The *Verhandlungen* of the *naturforschender Verein* in Brünn were in fact generally known and quite widely distributed, and Mendel's two papers were cited by Hermann Hoffmann in 1869, as well as by Focke later.[3] As for a lack of interest in the subject of plant

1. Bentley Glass, "A Century of Biochemical Genetics," *Proc. Am. Phil. Soc., 109* (1965), 227-236; quotation from p. 227.

2. Bentley Glass, "The Long Neglect of a Scientific Discovery: Mendel's Laws of Inheritance," in *Studies in Intellectual History,* by George Boas et al. (Baltimore: Johns Hopkins Press, 1953).

3. Hermann Hoffmann, *Untersuchungen zur Bestimmung des Werthes von Species und Varietät: Ein Beitrag zur Kritik der Darwin'schen Hypothese* (Giessen,

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breeding, Nägeli's reputation and active hybridization between species and Darwin's own great interest and example seem sufficient refutation. Darwin, in fact, cited Hoffmann's own crosses with radishes and beans in 1868 and 1876. It seems to me that it is far more probable that the minds of the plant-breeders and hybridizers, British, German, and French alike, were so obsessed by the overwhelming interest in the origin of species that they were concerned only with the crosses and hybrids *between* species. That obsession may in part account for Nägeli's neglect of Mendel's discovery, since Nägeli clearly paid little or no attention to results obtained by crossing simple varieties of garden peas, whereas he expressed to Mendel much interest in his interspecific *Hieracium* crosses, which came to nothing. Nägeli's own interspecific hybridizations evidently convinced him that heredity is a very complex business, to explain which he developed a "mechanisch-physiologische Theorie der Abstammungslehre."[4] Simple ratios, such as Mendel found in peas, could hardly explain the differences between species. Even if real, they probably lacked, he must have thought, any significant generality. One is led to wonder whether indeed Mendel's principles would have received such quick and universal acceptance in 1900 had it not then been demonstrated independently by Hugo de Vries, Carl Correns, and Erich von Tschermak that they applied to many hereditary characteristics in a number of species of plants.

Miescher's work met a somewhat different fate. It was widely known among chemists of the time (1869–1872). As soon as the chromosomes were identified as the bearers of hereditary properties, Miescher's nuclein (= nucleic acid) was shown to be a component of the chromosomes. By the year 1896, when E. B. Wilson wrote the first edition of his famous textbook *The Cell in Development and Inheritance*, he, like many others, was tempted to see in the nucleic acid the "formative centre of the cell."[5] A year earlier, Wilson had in fact said, after identifying nuclein incorrectly with a combination of nucleic acid and protein (albumin): "And thus we reach the remarkable conclusion that inheritance may, perhaps, be effected by the physical transmission

1869); Wilhelm Olbers Focke, *Die Pflanzenmischlinge, ein Beitrag zur Biologie der Gewächse* (Berlin, 1881).

4. Carl von Nägeli, *Mechanisch-physiologische Theorie der Abstammungslehre* (Munich and Leipzig: R. Oldenbourg, 1884).

5. E.B. Wilson, *The Cell in Development and Inheritance* (New York: Macmillan, 1896), p. 247.

of a particular chemical compound from parent to offspring."[6] In the third and last edition of *The Cell* (1925), Wilson had lost this hope entirely, and remarked only that nucleic acids seemed to be "remarkably uniform", except, erroneously, in so far as plants possessed RNA whereas animals possessed DNA.[7] Undoubtedly the widely accepted tetranucleotide theory of P. A. Levene and associates had blinded everyone to the variability of polynucleotides, since according to that view the four kinds of nucleotides present in nucleic acid occurred in equal numbers and in a regular sequence. Thus, three-quarters of a century passed before Miescher's work became fully appreciated and the new era of biochemical genetics began.

It would probably have seemed to anyone, in the first decade of this century, that Garrod's work would have avoided the prolonged eclipse meted out to both Mendel and Miescher. He was a highly distinguished British physician, and his studies were recapitulated and extended in the reports of them which formed his Croonian Lectures of 1908. These were published in 1909, and a second edition was issued in 1923.[8] Garrod took two big steps at once; first, that from the mutant gene to a particular blocked step in metabolism: second, that from the particular blocked reaction to the deficiency of a specific enzyme controlling it. Albinism, alkaptonuria, cystinuria, and pentosuria alike confirmed the conclusions he drew, regarding the first step; but the clinching demonstration that in each case a specific enzyme was deficient was not supplied until much later. Now many geneticists, both before 1908 and subsequently, had pointed to some important connection between genes and enzymes, although not so explicitly as Garrod. J. B. S. Haldane, of all geneticists in the first part of the century the best biochemically trained, nevertheless cited Garrod's work, in *New Paths in Genetics* (1942), only as affording examples of human inheritance of metabolic abnormalities as simple recessives.[9] He scarcely hinted at the significance of the existence of specific blocked steps in metabolism, and even less at any relation to specific enzymes. At the time he seemed far more interested in the probability that anthocyanin

6. E.B. Wilson, *An Atlas of the Fertilization and Karyokinesis of the Ovum* (New York: MacMillan, 1895), p. 4.

7. E.B. Wilson, *The Cell in Development and Heredity,* 3rd ed. (New York: Macmillan, 1925).

8. A.E. Garrod, *Inborn Errors of Metabolism* (London: Frowde, 1909); 2nd ed., London: Hodder & Stoughton, 1923.

9. J.B.S. Haldane, *New Paths in Genetics* (New York and London: Harper & Bros., 1942).

pigments in flowers and antigens in vertebrates might be direct products of the genes, although he said also: "It is not unreasonable to expect that enzymes will be found among the immediate products of gene action." [10] That seems to be the only mention of enzymes in the book.

George Beadle summed up the situation neatly in his Nobel laureate address in Stockholm in 1958.

> In this long and roundabout way, first in Drosophila and then in Neurospora, we had rediscovered what Garrod had seen so clearly so many years before. By now we knew of his work and were aware that we had added little if anything new in principle. We were working with a more favorable organism and were able to produce, almost at will, inborn errors of metabolism for almost any chemical reaction whose product we could supply through the medium. Thus we were able to demonstrate that what Garrod had shown for a few genes and a few chemical reactions in man, was true for many genes and many reactions in Neurospora. [11]

Recently, in *Scientific American*, Gunther Stent has suggested that a fourth case should be added to the annals of long neglect of highly important pioneering studies in biology. [12] For this dubious honor he nominates the work of Avery, MacLeod, and McCarty, published in 1944, which described their effort to determine the chemical nature of the material responsible for transforming pneumococcus cells from the

10. Ibid., p. 60.

11. George W. Beadle, quoted from H. Harris, *Garrod's Inborn Errors of Metabolism* (London: Oxford University Press, 1963), p. 50.

12. Gunther S. Stent, "Prematurity and Uniqueness in Scientific Discovery," *Scientific American, 227* (Dec. 1972), 84-93.

In the column "The Authors" in this same issue of *Scientific American,* p. 11, Stent is quoted in regard to the genesis of this article, as follows:

"In May of 1970 the American Academy of Arts and Sciences held a small conference in Boston on the History of Biochemistry and Molecular Biology at which I was asked to make a few brief comments following an account by Salvador Luria of the origins of molecular genetics. I intended to speak for about five minutes, a time I thought was more than enough to point out the relevance of the prematurity and the uniqueness concepts to Luria's reminiscenses about Oswald Avery and James Watson. But the conference participants – both scientists and historians – kept on interrupting me, and my 'brief comments' eventually lasted twice as long as Luria's lecture. This article is the product of that vigorous discussion, and among the discussants I am especially indebted to Robert K. Merton and Harriet A. Zuckerman of Columbia University for helping me to focus my ideas more sharply."

The *Records of the American Academy of Arts and Sciences* for 1971-72, p. 24, contain a brief report on the conference referred to. It states that the transcript of the proceedings was completed in the fall of 1971, but was not published, although a large number of copies were distributed to participants and other interested persons. I have not had access to that transcript.

avirulent (rough colony) type to the virulent (smooth colony) type when the former were treated with extracts from dead cells of the latter type.[13] The phenomenon was first discovered by Griffith in 1928, and Avery and his associates had been laboriously and patiently studying it during the ensuing sixteen years. At the end of that time they were able to say that the transforming material was, with high probability, deoxyribose nucleic acid (DNA).

Of general significance is Stent's suggestion of a criterion of *prematurity* that he regards as responsible for the failure to appreciate, at the time of discovery and for a long subsequent period, such works as Mendel's, Miescher's, and Garrod's. The criterion may well be valid even though Stent's fourth example of long neglect may not be well chosen. Also, the criterion may be valid, yet not account entirely for the long neglect that occurs. These seem to be very worthwhile matters to consider.

Stent indicates that the genesis of his study of the reception by geneticists of the work of Avery, MacLeod, and McCarty went back to "a brief retrospective essay on molecular genetics, with particular emphasis on its origins," which he published in 1966[14] and for which he was taken to task because he failed to mention the definitive work of Avery and his associates. Stent continues, in his *Scientific American* article, as follows:

> I was taken aback by this letter and replied that I should indeed have mentioned Avery's 1944 proof that DNA is the hereditary substance. I went on to say, however, that in my opinion it is not true that the growth of molecular genetics rests on Avery's proof. For many years that proof actually had little impact on geneticists. The reason for the delay was not that Avery's work was unknown to or mistrusted by geneticists but that it was "premature". . . . By lack of appreciation I do not mean that Avery's discovery went unnoticed, or even that it was not considered very important. What I do mean is that geneticists did not seem to be able to do much with it or build on it. That is, in its day Avery's discovery had virtually no effect on the general discourse of genetics.

What, then, does Stent mean by the *prematurity* of a piece of scientific work?

> . . . there is such a criterion. A discovery is premature if its implications cannot be connected by a series of simple logical steps to canonical, or generally accepted, knowledge.

13. Oswald T. Avery, C.M. Macleod, and Maclyn McCarty, "Studies on the Chemical Nature of the Substance Inducing Transformation on Pneumococcal Types," *J. Exp. Med.*, *79* (1944), 137-158.

14. G.S. Stent, "Introduction: Waiting for the Paradox," in *Phage and the Origins of Molecular Biology,* ed. by John Cairns, Gunter S. Stent, and James D. Watson (Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory of Quantitative Biology, 1966), pp. 3-8.

I believe that this is a very important hypothesis about scientific work, and deserves a full and careful examination. Stent believes that the persistent influence of Levene's tetranucleotide theory was the reason Avery's demonstration – that DNA is the transforming principle in the alteration of pneumococcus organisms from rough to smooth (avirulent to virulent) – was not heeded. It was not until 1950, as I pointed out in my paper already cited and as Stent also emphasizes, that the tetranucleotide theory was overthrown by the increasing weight of evidence against it obtained by Chargaff from the analysis of nucleic acids from different species. [15] Stent therefore insists that for geneticists the real demonstration that DNA is the actual physical basis of heredity was the classic experiment of Alfred Hershey and Martha Chase in 1952. [16] And so it was; but Stent has not told the whole story. We may well begin by asking: why, indeed, did Hershey and Chase undertake their famous experiment? Was it not because, by 1950, not only the demise of the tetranucleotide theory but also the implications of the work on the pneumococcus transforming principle were sufficiently evident? Someone had to try to devise a critical experiment that would answer the great question.

In his recent article in *Scientific American*, Stent uses the volume of essays from the symposium held on the fiftieth jubilee of genetics, *Genetics in the Twentieth Century*, [17] as a basis for his deductions about the failure of the leaders of genetics to appreciate the significance of Avery's work on the transforming principle. The point is well made. Except for Mirsky's devaluation of the biochemical evidence on the ground that purification may not have been complete and that some tiny fraction of protein may have remained with the DNA that transformed the rough into smooth pneumococci, we find only the briefest references to Avery's work, one by Beadle, the other by Lederberg. To quote Stent:

> Yet Mirsky's essay is the only one of the twenty-six in which the implications of that work are discussed. (Lederberg's Golden Jubilee essay refers to it briefly as a promising development in bacterial genetics, and Beadle's essay devotes two sentences to it, saying that it "has certainly introduced another chapter in genetics,

15. E. Chargaff, "Chemical Specificity of Nucleic Acids and Mechanisms of Their Enzymatic Degradation," *Experientia, 6* (1950), 201-209.

16. A.D. Hershey and Martha Chase, "Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage," *J. Gen. Physiol., 36* (1952), 39-56.

17. L.C. Dunn., ed., *Genetics in the Twentieth Century: Essays on the Progress of Genetics during its First Fifty Years* (New York: Macmillan, 1951).

and one that promises to be among the most exciting. It has given chemists new incentive to learn about the nucleic acids, compounds which everyone recognizes to be extremely important biologically and about which so little is known."

Nevertheless, before we accept Stent's hypothesis of prematurity as applicable here, it is incumbent upon us to scrutinize a few other documents more explicitly. One such, a highly important volume to which Stent has not referred, is the Cold Spring Harbor Symposium for 1946, entitled *Heredity and Variation in Microorganisms.*[18] This is critical to our considerations for several reasons. In the first place, it marked the resumption of the Cold Spring Harbor symposia after several years of wartime lapse, and the subject chosen shows that during World War II an entirely new and rapidly advancing field, the genetics of microorganisms, had become of major importance in genetics. This symposium volume was the first striking evidence of that fact. Held just two years after publication of the paper by Avery, MacLeod, and McCarty, the symposium is particularly interesting because, of the twenty-seven papers presented, four were concerned with bacterial viruses (bacteriophages). One of these was contributed by Alfred D. Hershey. As Stent well knows, not only was Cold Spring Harbor a world center in the development of the earliest phases of bacterial genetics, it was the true birthplace of phage genetics.

One paper presented at the symposium was "Biochemical Studies of Environmental Factors Essential in the Transformation of Pneumococcal Types," and the authors were M. McCarty, Harriett E. Taylor, and O. T. Avery. Inasmuch as, unlike Gunther Stent, I had the good fortune to be one of the participants in the symposium, I can personally testify that the paper was received with the very greatest interest and was widely discussed among us. If my memory does not play me false, the reaction among geneticists was about as follows. The demonstration that the transforming principle is DNA is very strong, although purification is not yet so complete that everyone is convinced that some protein does not remain in the preparation. (Mirsky was not present and could not have been directly responsible for this opinion.) We must recognize, it was said, that only a single gene need be transferred to transform the rough cells to smooth, and the presence of a single protein gene in the partially purified material cannot be firmly excluded.

18. *Heredity and Variation in Microorganisms,* Vol. XI (1946) in the Cold Spring Harbor Symposia on Quantitative Biology includes "Biochemical Studies of Environmental Factors Essential in Transformation of Pneumococcal Types," by M. McCarty, Harriett E. Taylor, and O.T. Avery, pp. 177-183.

Inasmuch as suspended judgment is considered to be a great scientific virtue, we should suspend judgment. Opposing this view was evidence, presented in the paper by McCarty, Taylor, and Avery (as well as in others by McCarty and by McCarty and Avery published in 1946),[19] that deoxyribonuclease rapidly destroys the transforming principle, but that it is not affected by protein denaturation or precipitation procedures or by action of proteases.

In the Cold Spring Harbor Symposium for 1951,[20] Rollin Hotchkiss and Harriett Ephrussi-Taylor separately summed up developments at that time in further studies of the transforming principle, including these points: (a) further chemical purification and exclusion of protein from the preparations; (b) work done on transformation of other genes, namely, penicillin resistance in pneumococcus; (c) this new transformation also inactivated by deoxyribonuclease; and (d) discovery of a *Salmonella*-transforming preparation by Lederberg, Lederberg, Zinder, and Lively (reported at the same symposium) and of a transforming principle in *Hemophilus influenzae* (reported by Zamenhof in discussion following the paper by Harriett Ephrussi-Taylor). Ephrussi-Taylor devoted considerable attention to discussion of the question whether the transforming agents are genetic units.

It thus becomes apparent that in the seven years following the original publication by Avery, MacLeod, and McCarty a very active program proceeded on the nature of the transforming principles in bacteria. It was sufficient to demonstrate to geneticists that the transformations are a general phenomenon and not a peculiarity of a single character in *Pneumococcus*; that the agent is in all probability DNA; and that the transforming agents are equivalent to other genetic units (genes) in bacteria.

19. M. McCarty, "Purification and Properties of Desoxyribonuclease Isolated from Beef Pancreas," *J. Gen. Physiol., 29* (1946), 123-139. M. McCarty and O.T. Avery, "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types. II. Effect of Desoxyribonuclease on the Biological Activity of the Transforming Substance," *J. Exp. Med., 83* (1946), 89-96. M. McCarty and O.T. Avery, "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types. III. An Improved Method for the Isolation of the Transforming Substance and Its Application to Pneumococcus Types II, III and VI," *J. Exp. Med., 83* (1946), 97-104.

20. *Genes and Mutations,* Vol. XVI in the Cold Spring Harbor Symposia on Quantitative Biology (1951), includes "Transfer of Penicillin Resistance in Pneumococci by the Desoxyribonucleate Derived from Resistant Cultures," by Rollin D. Hotchkiss, pp. 457-461; "Genetic Aspects of Transformations of Pneumococci," by Harriet Ephrussi-Taylor, pp. 445-456; and "Recombination Analysis of Bacterial Heredity," by J. Lederberg, E.M. Lederberg, N.D. Zinder, and E.R. Lively, pp. 413-443.

When we recall that Hershey and Chase attended these symposia and participated in the discussions, and that Cold Spring Harbor was the focus of the genetics of microorganisms during these eventful years, it is scarcely surprising that the definitive experiment emerged in those same laboratories in due course of time. Was there, then, an unreasonable delay, occasioned by the prematurity of Avery's discovery? I think not, unless one adopts the indefensible position that Avery's discovery was itself definitive. It took even the rapidly developing fields of bacterial and phage genetics those intervening years to solidify the idea and to develop the techniques for refined genetic analysis. Had there been more manpower in those fields at the time, events might have moved much faster; but this was not the period of the fifties. It was the nineteen-forties, when pure science was just beginning to recover from the interruptions and delays occasioned by wartime.

Thus I come to a different conclusion. Contrary to Stent, I do not think it correct to put the work of Avery and his associates in the category of really premature work, long delayed in attaining recognition. Much depends upon the general rate of advance, and in the forties the pace was not so swift. The eight years between 1944 and 1952 really represented rapid progress in the development and consolidation of the evidence about the nature of the transforming principle. Moreover, when the issue is one of upsetting what Thomas Kuhn[21] would call a major paradigm in a science, viz., the revolutionary replacement of protein as the physical basis of heredity by the virtually unknown and neglected nucleic acids, there must be critical evidence, definitive experimentation, and a sufficient warrant to generalize. Especially the last of these was, I believe, slow in coming. It required that the transforming agent be demonstrated to apply to more than one character and to more than one species – in other words, that it was clearly to be identified with the genes of higher organisms. There was great doubt about that in the minds of many geneticists in the mid-forties. Indeed, did bacteria have genes and chromosomes, like those of multicellular plants and animals? They revealed heredity of many characteristics, and mutation, both spontaneous and induced, was abundant. The Luria-Delbrück analysis of mutation[22] was, however, only three years old at the time of the 1946 symposium; and the demonstration of sexual

21. Thomas S. Kuhn, *The Structure of Scientific Revolutions* (Chicago: University of Chicago Press, 1962).

22. S.E. Luria and M. Delbrück, "Mutations of Bacteria from Virus Sensitivity to Virus Resistance," *Genetics, 28* (1943), 491-511.

recombination in bacteria, which was to open up so many fruitful avenues of discovery in bacterial genetics, was announced at the very same 1946 symposium in which the paper by McCarty, Taylor, and Avery was presented and discussed. Much had to be done in the five years before the second Cold Spring Harbor Symposium on the subject paved the way for the Hershey-Chase experiment.

To conclude, one may say that the criterion of prematurity, as defined by Stent, without question applies well to the classic cases of neglect of the work of Mendel, Miescher, and Garrod. It leads, on the contrary, to the rejection of the proposition that the work of Avery, MacLeod, and McCarty should be added to their number. The analysis also suggests strongly that another criterion should be added to the determinative process, that of a *lack of generality*. Mendel's peas were thought to be unsuitable material for studying the heredity of species differences, and his laws were not clearly applicable to the other plants he attempted to use. Miescher was limited by his use of pathological material (pus cells) and reproductive cells (salmon sperm). Later interpretations were led astray by the isolation of RNA from yeast cells in contradistinction to DNA from animal cells, whence it was supposed, on the basis of a false generalization, that this difference characterized plants and animals, respectively. Garrod had the misfortune to make his discoveries in the clinic, and everyone knew that human heredity was not amenable to experimental analysis by breeding and was not quite respectable for a genuine scientist to pursue. The long neglect lasted respectively thirty-five years, seventy-five years, and thirty-six years, roughly, for Mendel, Miescher, and Garrod. For the work of Avery and his associates, I believe there was far less prematurity and that the delay of eight years, or less, was occasioned mainly by doubt about its generality. Had Griffith, on the other hand, not only shown the existence of pneumococcus transformation in 1928, but also at that time demonstrated that the material responsible was DNA, undoubtedly the discovery would have been premature and would have received no more attention than it did until the later demonstration of the generality of the phenomenon and the demonstration by Chargaff of the non-validity of the tetranucleotide theory.