CORRESPONDENCE

Avery in Retrospect

SIR,—Dr H. V. Wyatt has drawn attention to the muted manner in which Avery, MacLeod and McCarty expressed themselves in 1944 on the significance for genetics of their work on the transforming principle of pneumococcus[1]. He has exposed the tendency of later commentators to read more "knowledge" into the statements of "information" than is perhaps justified. At the same time it is only fair to the three Rockefeller scientists to state Avery's reasons for the narrow delimitation of this work and for the non-committal discussion of its significance. These features can be highlighted by a comparison of the 1944 paper with papers written by André Boivin and his collaborators who worked first at the Pasteur Institute then at the University of Strasbourg.

In May 1943 O. T. Avery wrote to his brother Roy a famous letter from which Dr Wyatt quotes the phrase "Sounds like a virus—may be a gene". Avery then added, as if hastily: "But with 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 bristles with implications"[2]. He went on to assure his brother that a lot of well documented evidence was needed before anyone could be convinced that protein-free DNA had the properties he claimed. This was the task he undertook. Just how the DNA acted was a separate question, the answer to which would clarify the biological significance of transformation. In this connexion it should be borne in mind that although Robinow had demonstrated nuclear structures in rod-shaped bacteria in 1942[3], no case of conjugation in bacteria had been reported in 1944, and it was not until 1946[4] that Lederberg and Tatum had good evidence of bacterial recombination. Furthermore Avery had to contend with the traditional interpretation of bacterial transformation as given by Neufeld and Levinthal in 1928[5], Dawson in 1930[6], Alloway in 1933[7]—that the recipient cells have retained the power to elaborate the capsular polysaccharides of several types of pneumococci and need only the stimulus of the transforming principle, this being specific for the development of only the donor type coat. The 1944 interpretation was an advance in this position.

It was the enzyme studies which formed the bulwark of Avery's case and accordingly his efforts with McCarty, after the 1944 paper, were directed at improving this evidence[8]. Here it must be conceded that trypsin and chymotrypsin alone are inadequate as agents to remove all possible types of protein from the transforming substance. Unfortunately they could not use pepsin because the DNA was damaged at the *p*H required for its action[9]. Here pronase, had it been known at that time, could have filled the gap. Also none of these enzymes effectively digests protein until it is denatured, hence it was possible for Mirsky to find a weak point here when he attacked the evidence from enzymology in 1947[10].

In contrast to Avery, André Boivin gave all too few details of his work with Vendrely and Lehoult at the Pasteur Institute on the transformation of *Escherichia coli* types. At a meeting of the Academie des Sciences in November 1945 these workers claimed to have obtained results like Avery's but using *E. coli*[11]. In 1942, stimulated by the work of Dawson, Sia and Alloway, they had tried to effect transformation of types in *E. coli* and before seeing the 1944 paper of the Rockefeller scientists they had come to the conclusion that the transforming principle was a nucleoprotein. When they learnt of this work they removed the protein from their nucleoprotein autolysates of *E. coli* and found the nucleic acid residues still capable of transformation. At the time this work was regarded as an extension of the pneumococcus case to another bacterial species. Only later was doubt thrown on Boivin's work after subsequent attempts to reproduce it failed[12].

To the historian it is of interest that Boivin was prepared to go much further than Avery in his interpretation of the work. The title of his paper in *Experientia* contains the phrase "Significance for the Biochemistry of Heredity". In the conclusion the discovery of the identity of the transforming principle is described as opening "new horizons", promising for the biochemistry of heredity. "In particular, it is on the side of the nucleic acid and not at all on that of the protein of the nucleoprotein macromolecule constituting a gene that one must find the basis for the inductive properties belonging to the gene"[13]. When Boivin attended the Cold Spring Harbor Symposium on "Nucleic Acids and Nucleoproteins" in June 1947, he gave a remarkable paper[14] in which he related the work on bacterial transformation to Beadle and Tatum's work on biochemical genetics, described Tulasne's confirmation of Robinow's work on the bacterial nucleus[15], and the chemical mechanism involved (Vendrely and Lipardy[16]), and gave Tulasne's and Vendrely's cytochemical evidence, using RNAse and DNAse, for the localization of RNA in the bacterial cytoplasm and DNA in the nucleus of *E. coli*[17].

When we look back over the mass of literature in the 1940s it seems scarcely possible that André Boivin could have so accurately predicted the structure which the nascent subject of molecular genetics was to take. Consider the following statement[14]: "We may, at the most, catch a glimpse of a series of catalytic actions which set out from primary directing centres (the deoxyribonucleic genes) proceed through secondary directing centres (the ribonucleic microsomes-plasma-genes) and thence through tertiary directing centres (the enzymes), to determine finally the nature of the metabolic chains involved, and to condition by this very means, all the characters of the cell in consideration . . .".

Although the Avery, MacLeod and McCarty paper was published in a journal with a fairly limited readership, the subsequent papers in New York, Atlantic City, Hershey (Pennsylvania), and Cold Spring Harbor in 1946, and in the latter again in 1947, brought bacterial transformation to the attention of a wide audience. In addition Luria, Dobzhansky and Burnet visited Avery personally in the 1940s. In war-torn Europe conditions were not conducive to the public discussion of Avery's work, yet in Paris André Lwoff and Boris Ephrussi held a colloquium with support from the Rockefeller Foundation at which the new work which had had its genesis in Avery's discovery was reported.

This new work concerned the demonstration that bacterial transformation was not confined to one hereditary characteristic and that DNA did have the properties required of the hereditary substance. When we see Hotchkiss's and Chargaff's evidence against the tetranucleotide hypothesis, Chargaff's demonstration of the species specific base composition of DNA, and the Boivin Vendrely Rule governing the DNA content of diploid and haploid cells as the fruit of work initiated by the

Avery, MacLeod, McCarty discovery, it is no longer possible to maintain that their paper was either ignored or unknown. When we see how little was known about genetic processes in bacteria and the chemistry of DNA in 1944 compared with 1950, Avery's caution can be seen as justified. As for the geneticists, it is clear that what caught their imagination was not the identity of the transforming principle—whether it was nucleic acid or nucleoprotein did not mean a great deal to them—but the possibility, at last, to bring about a given hereditary change by a specific treatment. Hence the reason for the widespread habit of referring to bacterial transformation as "directed mutation".

H. J. Muller was exceptional among geneticists in being concerned about the chemical identity of Avery's transforming principle, but was impressed by Mirsky's opinion. To Darlington he wrote: ". . . Mirsky gave reasons for believing that Avery's so-called nucleic acid is probably nucleoprotein after all . . ."[18]. Yet again, what attracted Muller was the possibility of fitting transformation into the grand scheme of cytogenetics. In the transforming substance, he suggested, there were chromosomal fragments consisting of nucleoprotein, which were incorporated into the genetic apparatus of the recipient bacterium in transformation.

As in the case of Mendel's paper, the scientists of a given period found in Avery's paper what they were looking for, but unlike that earlier case, the 1944 paper was not ignored or unknown. It posed questions about DNA which by 1950 could be answered. What Avery failed to say, Boivin said, but his brave words did not profoundly alter the climate of opinion until the Boivin Vendrely Rule was established[19]. Only with the advantage of hindsight can we see the significance of the 1944 paper as obvious. Only by confining our attention to the published record and the citation statistics on Avery's paper can we arrive at the view that it was little known or undervalued.

Yours faithfully,

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