The Eighth Day of Creation

Makers of the Revolution in Biology

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1979

Simon and Schuster • New York

four carbon atoms, shares two adjoining carbon corners with the pentagon, which has another pair of nitrogen atoms in it. Guanine and adenine differ only in small side groups attached to other corners of the hexagon. These two bases are called purines because of their chemical relationship to uric acid and so to urea—and biochemistry is conventionally said to have begun with the synthesis of urea, by Friedrich Wöhler, in 1828. The three other bases, thymine, cystosine, and uracil, 1893, 1894, and 1900, are called pyrimidines, a longer name of unilluminating origin but a smaller, simpler structure: a single ring, just the same hexagon of two nitrogen and four carbon atoms—with side groups, again, that make the differences.

By the 1920s, it was realized that there are two kinds of nucleic acid. In one, now called ribonucleic acid, RNA, the bases are adenine and guanine, cytosine and uracil. In the other, the ribose sugar lacks a fringe oxygen atom—hence deoxyribose nucleic acid—and a pyrimidine has been switched, uracil replaced by thymine. Uracil had first been found in yeast, and was known in a species of wheat. Thymine had been discovered in calf thymus gland, whence its name, and was known in every animal cell where it had been looked for. Uracil and thymine are very similar. For a while it was thought, then, that ribonucleic acid, bases G A C *U*, was for plants and deoxyribonucleic acid, G A C *T*, was animal. This idea collapsed in the early thirties under accumulating evidence that both RNA and DNA are universal. By then, too, it was known that the chromosomes are in large part DNA. Nonetheless, DNA was thought to be built up in the simplest way imaginable, with the nucleotides following one another in fixed order in repeated sets of four. This exceedingly elementary picture was called the tetranucleotide hypothesis. It was propounded by Phoebus Aaron Levene, at the Rockefeller Institute for Medical Research, an organic chemist of highest reputation. Accurate measurement of the proportions of the bases in samples of DNA was impossible with the chemical techniques available. And so the belief was held with dogmatic tenacity that the DNA could only be some sort of structural stiffening, the laundry cardboard in the shirt, the wooden stretcher behind the Rembrandt, since the genetic material would have to be protein.

Rigorous proof that the gene is DNA and not protein appeared in 1944, when Oswald Avery and fellow workers at the Rockefeller Institute in New York published a paper in *The Journal of Experimental Medicine* about inheritable transformations that occur in a strain of pneumonia bacteria when they are mixed with DNA extracted from a different strain. Avery's paper is today universally cited as fundamental, always with the reservation that the proof took years to be credited. In February 1944, when the paper appeared, Crick was working for the British Admiralty as a physicist, designing naval mines, and Watson was a precocious college boy in Chicago, consumed by ornithology the way another might have been absorbed in railway timetables. When they met, seven years later, both knew of Avery's work, though then and for several years more it was still generally believed and widely asserted that genes are protein.

On the point of picking up Avery's paper, I realized that for the moment

I was surfeited with abstractions about DNA. I wanted to know what the stuff looks like, how it is prepared, something of how one tells that it is not protein. In the entrance stairwell of Perutz's laboratory building, before a floor-to-ceiling window, I had stopped to look at a molecular model, eight feet tall, of the double helix of DNA, and stopped again, on the landing above, at a model, nearly as large, that claimed it was of the alpha helix, a structure in proteins discovered by Linus Pauling. Surely, I thought, the two models could be told apart if one knew what to look for. The stretch of protein wound up and around, linked back and forth to itself by banisters that were jarringly off the perpendicular, bobbing and weaving upwards in a boxer's shuffling syncopated rhythm, while the DNA, right enough, was double, the strands separated by alternating narrow and wide grooves connected not by balusters but by the horizontal bases, planes laid flat to form a slowly revolving series. One could say the DNA had a calmer, cooler architecture. But really it was preposterous, despite the monumental use to which these models had been put, to think of them as aesthetic objects. The overwhelming impression that each gave was much the same: a visually confusing lacework of thin rods intersecting at knobs colored variously blue, red, black, white. This DNA was still an abstraction. Feeling exceedingly elementary, I asked Sidney Altman, a friend and molecular biologist now at Yale, to show me what DNA really is.

The afternoon I arrived at Altman's laboratory, he produced a large bottle of a gray liquid that looked like dishwater. "We start with this. Bacteria in a culture medium—just water, some salts and a carbon source, and some amino acids they need. They've been growing since I started them last night. That's why it's so cloudy: you're seeing a mist of bacteria—not the bacteria themselves but the turbidity they cause by scattering the light. You realize, the chief thing you're going to learn is how easy things are once you know how to do them. Now we centrifuge them down, to concentrate the bacteria." He poured the gray liquid into four stubby plastic test tubes, which we took over to a large box, like a stainless-steel washing machine, with a top hatch that lifted heavily. Within was a pear-shaped spindle with slanting holes like spokes. Altman put the tubes into the holes and set the machine's speed control at 8,000 rpm. "Come back in half an hour."

When Altman took out the tubes, the liquid had turned water-clear, while at the bottom of each was a small heap, in color the pale yellow-gray of an old nylon shirt. "This is ersatz science," he said. "We're not doing this for any real experimental purpose. Takes the edge off one's precision. The cells are all in those pellets. We can throw out the supernatant." He poured away most of the liquid. Then at his bench, he scraped up the pellets of bacteria with the end of a glass rod and transferred them all to one tube with a little liquid. "They're back in suspension but concentrated a hundredfold." Indeed, the liquid, clear a moment earlier, looked filthy. He turned to an appliance the size and shape of a melon, with a large rubber navel on top that began to shake with silent laughter as he pressed the end of the tube to it. "Breaks up the clumps." In a burlesque of the classic gesture of the

movie scientist, Altman held up the tube to the light as he added a liquid from another bottle. "This is EDTA—ethylenediaminetetra-acetate—which takes up the magnesium from the bacterial cell walls. Bacteria have tough cell walls, but this stuff makes them very weak." He searched a shelf, found a plastic jug labelled "10% SDS," added some of that. "Sodium dodecyl sulfate—all it is is a detergent; you could wash dishes with it. We put it in to solubilize the cell walls. The idea is to break the cell walls to get the DNA out." The technical term for rupturing cell walls is "lysis." To lyse cells, biologists use strong chemicals, or even grind the cells in a mortar with sand; animals get the same results with the subtler means of an enzyme, called lysozyme, present in such body fluids as tears, saliva, and intestinal mucus, which has the protective effect of breaking open bacteria that attempt to invade. Altman put a black rubber stopper into the tube. There was about a quarter cupful of liquid in it. "As the cells lyse, the bacteria will vanish and the mixture will begin to clear. At the same time it will get very viscous, because the DNA in each cell is essentially one extremely long molecule, and these are freed. Like a basketball player getting out of a Volkswagen, only more so." He set the tube half into a fish tank full of running water, where a thermometer said 37° C., blood heat. "Come back in half an hour."

Out of the warm-water bath, the tube of liquid was clear again. "Now we add phenol—what used to be called carbolic acid; our grandmothers used it to disinfect drains. The phenol attacks the protein, which is why it worked for grandmother, but it leaves the DNA alone. And it's heavier than water, so it will sink to the bottom with the protein, while the nucleic acids stay in the aqueous phase at the top." He put the stopper back into the tube, started gently rocking it. The liquid was bubbling slightly and began to look pale gray and thick, like sputum. "Doing this by hand keeps the DNA from breaking so much. Those long fibres, once they're floating free, are exposed to a lot of shearing force. That disgusting glob of white is the protein. In a minute we'll centrifuge them apart." This time he used a small machine standing on his bench, mushroom-shaped, of gray metal. First he weighed the tube, and filled a second one with which to balance the machine. A hand-lettered sign on the centrifuge said "*four buckets are hot—beware.*" As he closed the lid, the spin was starting to tilt the tubes up into the horizontal plane. "Come back in half an hour."

As we waited for the spinning to stop, Altman handed me a small brown bottle. "This is what the stuff we're preparing would look like if you made a lot of it and dried it. It's purified DNA, as it happens from calf thymus glands, one of the traditional sources." The bottle was full of small bits of what looked like scraps from an old linen handkerchief, white and obviously fibrous. "They're a lot tougher than lint, though. Those long molecules lying together have a very high tensile strength. No, don't touch, even slight impurities can start breaking the molecules up." With tweezers, he took out the top flake, dropped it into a small vial with a screw lid. "Something to show your friends."

The tube from the centrifuge now had a layer of clear liquid at the bottom,

"the phenol," then a layer of white, "the protein—really goopy," and above that another layer of clear liquid. "The DNA is in that top layer. It gets so viscous we'll have trouble getting it out without contaminating it with some of the protein beneath. If this were real, I'd have to be a lot more careful." He packed the tube and assorted glassware into shaved ice in a battered plastic ice bucket. He took a wide-mouthed glass pipette and tried to suck up the layer of liquid at the top of the tube; every time he lifted the pipette slowly away, the liquid simply plopped back into the tube. "It's so viscous it pulls itself out again." He tried other pipettes, at last took a small one over to a bunsen burner, where he held the tip in the flame, and then bent a kink into it. With this he got the liquid up a few drops at a time, and into another, narrower tube. He tilted that. "Watch how it flows. That's *really viscous!* It's good stuff.

"Now, this last step is the spectacular one. I'm going to layer in twice as much ethanol, absolute alcohol." He poured the alcohol slowly down the side of the tube so that it floated, cream on Irish coffee. "Now we stir with this glass rod, gently winding. The alcohol precipitates the DNA, and we pull the fibres out of solution like winding spaghetti out of sauce." As he twisted the glass rod, the tip began to thicken with cobwebs, wet and translucent. As he lifted the rod out, the attached fibre pulled into a long filament. "Amazing. That's a lot of individual molecules lying together, of course. But you could use a fibre like that for X-ray analysis of its structure, the way Rosalind Franklin did."

The principle of DNA extraction is simple: break the cells open, get rid of protein by treatment with phenol or chloroform, precipitate DNA with alcohol. High-school pupils these days learn to extract DNA in science classes, though not, if their teacher is wise, from bacteria; and teacher scrambles to keep ahead with the aid of manuals and source books that tell how to go on to analyze the composition of the DNA by such techniques as chromatography or electrophoresis, in which absurdly small amounts of biological substances can be persuaded to separate themselves for identification and measurement as they migrate, in solution, down a sheet of filter paper or across a hard slab of gelatine, some travelling faster and farther than others because of differences in weight or solubility or electrical charge of the individual molecules.

Methods of such discrimination and finesse—of such chemical resolving power—were only beginning to be available when Avery published his surprising paper. Yet what he accomplished in the early forties is still respected as masterly. His was far from ersatz science. The paper is marked by the probing sensitivity with which he responded to what he did not know. The title is as arid as any in the literature: "Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III," by Oswald T. Avery, Colin M. MacLeod, and Maclyn McCarty. Within, the writing is excellent—supple and taut. Procedures come across vividly. Not just a short run of experiments is reported, but years of work and years of pondering. The argument is tough, clear, close-grained. Scientific papers in our day are written to an artificial, sterilized form; Sir

Peter Medawar—an immunologist who shared the Nobel Prize for physiology or medicine in 1960—has suggested that they are so deliberately antihistorical as to be a deception, for "They not merely conceal but actively misrepresent the reasoning that goes into the work they describe." Avery's great paper, though, shares with the classics of science of previous centuries at least one quality now grown rare: from the first paragraphs through to the end, one feels an original curiosity working.

Avery was by training a physician, as were his two associates in the paper. As specialisms then went, he was not a geneticist and not exactly a biochemist, but an immunologist and microbiologist. That is, he worked with microorganisms; and among them microbes rather than viruses, and among microbes the bacteria that cause pneumonia, with the long-term hope of developing sera with which to treat acute cases.

Microorganisms can go through as many generations in half a week as mankind has had since history began. Each generation for a bacterium is a doubling; many a virus multiplies by the fiftyfold or the hundredfold. The foods such creatures use are so simple that what goes into them can be exactly known, controlled, and compared with what they make of it. Further, it is easy to spot variations. A bacteriologist or a molecular geneticist can routinely select from a billion or more separate cells the single individual that possesses some particular inheritable trait. These and other advantages have made microorganisms the favorite subjects for many kinds of biology for the middle third of this century, especially for geneticists, though right now a change is taking place, back to fruit flies, mice, and other higher animals, because one-celled creatures are too simple, internally as well as in being one-celled, for the questions molecular biologists have come to. Yet thirty years of intensive scrutiny (as Watson points out to students) mean that next to man himself, the world's most thoroughly understood organism is his small companion through life, the normally benign intestinal bacterium *Escherichia coli.* Determining the results of an experiment with bacteria can be easier than one might suppose. Often enough a microscope is not even needed: just examine where the bugs are growing, on broth or gelatine in one of those fragile low-sided glass dishes, to see the colonies' size, color, texture.

By such simple and visible criteria, the world of Avery's *Streptococcus pneumoniae,* called pneumococcus, is divided into the Rough and the Smooth. S forms are virulent. They kill laboratory animals. A bacterium of S form surrounds itself with a plump, gelatinous capsule, which it builds not of protein but of a complex sugar, a polysaccharide. The capsule partly protects the bacterium from the defenses of the infected animal, and so always goes with virulence in pneumococci. R-form bacteria have lost their ability to make capsules and so to cause infection. (R forms are now understood to be mutants that fail to make the enzyme that knits together the capsular polysaccharide.) To get them, bacteriologists grow pneumococci in a medium made hostile to the ancestral S form. They are called R for no microscopic reason but because, Avery wrote, "On artificial media the colony surface is 'rough' in contrast to the smooth, glistening surface of colo-

nies of encapsulated S cells." R and S forms of pneumococci had first been distinguished in 1923, in London, by Frederick Griffith, a physician doing research in the Pathological Laboratory of the Ministry of Health. Variant virulent S types had also been found, and numbered I, II, III. These differ much less in what can be seen in a glass dish, but can be told apart with certainty by immunological tests. Antibody reactions are among the most exquisitely sensitive detection systems biologists possess. Tested against serum from the blood of rabbits that had survived infection and developed a high degree of immunity, protein from pneumococci betrays its presence, Avery wrote, in dilutions as high as one in fifty thousand, and the capsular sugar in dilutions of one part in six million. Avery himself had discovered the immune reaction to capsular polysaccharide—the first evidence that animals can make antibodies to something not a protein—also in 1923, in the course of the work by which his laboratory established the fixed differences among the S types of pneumococci.

Then, in 1928, Griffith in London had published a startling discovery. He had injected mice with two pneumococcal preparations at the same time: a small amount of a living culture of the R form, derived from Type II and proved to be not virulent by itself, together with a large amount of a dead culture of the S form of Type III—killed by heat, containing no living bacteria, and proved to be not virulent by itself. In short, two different types, one an R, was live but not virulent, and the other an S, virulent but killed. Many of the injected mice had died. In the heart's blood of these mice, Griffith had found living, virulent pneumococci, of the S form—and not Type II but Type III.

The change was permanent and inherited. Generations more of culturing had produced nothing but more Smooth, virulent Type III pneumonia germs. Other experiments produced similar transformations of other pneumococcal types. Griffith's discovery suggested doubts about the existence of distinct true-breeding species among bacteria. It opened grave practical problems for epidemiologists and immunologists. It raised clouds of speculative and spurious explanations. All in all, microbiologists found transformation of bacteria about as unsettling as atomic physicists, at that same time, were finding the transmutation of elements by interaction with neutrons and protons. Avery at first found it impossible to credit Griffith's paper. The findings seemed to overthrow his own fundamental demonstration of the fixity of immunological types. But bacterial transformation was confirmed that same year in Berlin and in 1929 was repeated at the Rockefeller Institute.

Two years after that, associates of Avery's found that they could do the same experiment leaving out the mice. They could achieve transformation by growing a culture of R form in a glass dish in the presence of heat-killed pneumococci of the S form. Several months later, James Lionel Alloway, again in Avery's laboratory, took the pursuit of the transforming agent one twist further. Alloway broke open the S-form bacteria to set their contents free, then passed the culture through so fine a filter that the shells, together with any unbroken cells, were removed. When this extract, free of cells,

was added to a growing culture of the R form, transformation took place. Further, when he added alcohol to the extract, he got a viscous, "thick syrupy precipitate."

Rollin Hotchkiss, who joined Avery's laboratory in 1935, recalled in a biographical memoir thirty years later that Avery's characteristic question was a quick, insistent "What is the substance responsible?" For the next decade, Avery was increasingly preoccupied with step-by-step purification of the transforming agent and its identification. In the beginning, transformation was an uncertain, delicately balanced phenomenon. "Many are the times we were ready to throw the whole thing out of the window!" Avery said to Hotchkiss. At the last, Avery was able to take a culture of pneumococci of an R form that had been attenuated from an S of Type II, thirty-six generations back (all the way back to the Crusades, on a human time scale), and add to it what he knew to be a highly purified DNA extracted from an S of Type III—and he got out, in the next generation, fully developed "large, glistening, mucoid colonies" of S Type III. These then remained stable through succeeding generations. Recalcitrant strains of bacteria had been tamed, finicky conditions of culture mastered. Avery's difficulty was no longer the transformation itself but to prove that it was caused by DNA and nothing else, despite the fact that DNA had not been identified in pneumococci before and in defiance of the universal conviction, his own conviction at the start, that DNA was a monotonous molecule and genes were protein. Avery was a small man, a bachelor all his life, smooth-faced and thin; he wore pince-nez. Various friends remember that he would pass his hands across his bald head when perplexed, that he rolled his own cigarettes, that he was fastidious with words and reserved with conclusions, that he was a gentle, versatile, overwhelming monologuist for whom the pneumococcus was the microcosm of biology. Hotchkiss wrote, "My personal notes of 1936 record that in one of his discourses on transformation, Avery outlined to me that the transforming agent could hardly be carbohydrate, did not match very well with protein, and wistfully suggested that it might be a nucleic acid!"

Throughout the paper of 1944, with immaculate caution, Avery, MacLeod, and McCarty speak of their substance as "the transforming principle." To get it, they grew virulent Type III pneumococci at blood heat in twenty-gallon vats of broth made from beef hearts, spun out the bacilli in an iced centrifuge, suspended them in brine, and brought the "thick, creamy suspension of cells" quickly to a temperature hot enough to kill the cells and to inactivate "the intracellular enzyme known to destroy the transforming principle" (an enzyme now called, with brisk inelegance, DNase). They then washed the cooked pneumococci in three changes of brine to remove capsular sugar as well as whatever protein would come away, extracted the bacteria by shaking them for an hour in a solution of bile salt to break the cell walls (and then threw away the cell residue), and reprecipitated the extract with pure grain alcohol.

"The precipitate forms a fibrous mass which floats to the surface of the alcohol and can be removed directly by lifting it out with a spatula," the

paper said. This was now washed several times with chloroform to remove protein, and suspended yet again. A digestive enzyme was put in to eat away any remaining capsular sugar. Removal of protein was repeated, "until no further film of protein-chloroform gel is visible at the interface." Pure grain alcohol was added again, "dropwise to the solution with constant stirring." At a concentration where the alcohol nearly equalled the extract, "the active material separates out in the form of fibrous strands that wind themselves around the stirring rod. This precipitate is removed on the rod and washed. . . . The yield of fibrous material obtained by this method varies from ten to twenty-five milligrams per seventy-five liters of culture"—or, at best, just under one hundredth of an ounce from twenty gallons of culture. The method of extraction, before the introduction of detergents and using chloroform rather than phenol, was heroically laborious.

Avery and his colleagues set out to show what their transforming agent was—and what it was not, which was harder. They devised tests with an almost obsessive ingenuity that makes the paper a model of reasoning from and about experiment. The understated iteration takes on rhetorical power. Standard qualitative tests for protein—for example, add a pinch of copper sulphate and see if the solution turns blue-violet—were negative; those for DNA, strongly positive. Chemical analysis found the elements in proportions—particularly the telltale ratio of nitrogen to phosphorus, 1.67 to 1 on average—which agreed closely with what DNA, with its nitrogenous bases and its phosphates, should show but which would have been different if, despite the extraction methods, much protein had remained.

They turned to enzymes. The specificity and speed of enzymes, the power of each to catalyze its own reaction intensively and nothing else, fits them for the burden of proving a biological negative. Pure, crystalline enzymes were just beginning to be available, in great part through the work at a sister unit of the Rockefeller Institute, in Princeton. Other enzymes of proven strength in crude form were obtained from rabbit bones, swine kidneys, and the mucus of dogs' intestines. Of these, enzymes known to digest proteins left the transforming principle intact. Those known to degrade RNA left the transforming principle intact. And those that ignored protein but attacked samples of known DNA destroyed completely the activity of the transforming principle. Avery and his co-workers complicated the enzymatic tests by adding selective chemical inhibitors and by exploring the subtle effects of temperature variations on enzyme activity. Results always agreed, they reported, with what happened in parallel experiments with known DNA.

They went on to immunological tests. These demonstrated that neither pneumococcal protein nor capsular polysaccharide was present in the transforming extract up to the extreme limit of sensitivity of the technique. They spun a sample of the extract on the ultra-high-speed centrifuge, and found that as it sedimented, "the material gave a single and unusually sharp boundary indicating that the substance was homogeneous and that the molecules were uniform in size and very asymmetric"; the result

matched with DNA from calf thymus. They tried electrophoresis, and found that as the molecules in a solution of the transforming principle were propelled by a weak electric current, they stayed together as one substance—and that this moved relatively fast, as nucleic acids do. They found that the transforming principle absorbed ultraviolet light at certain wavelengths to yield the same profile as nucleic acids. They found, and saved for last, that the transforming principle could demonstrate its transforming power in extraordinarily small amounts—down to "a final concentration of the purified substance of 1 part in 600,000,000" of the culture medium containing bacteria of R form.

Avery's concluding discussion is one of those precursors that can sometimes be looked back to in science, or for that matter in philosophy or economic theory or painting, where one seems to see an idea struggling to shake free from a net of previous conceptions. Strikingly,

> the substance evoking the reaction and the capsular substance produced in response to it are chemically distinct, each belonging to a wholly different class of chemical compounds.
>
> The inducing substance, on the basis of its chemical and physical properties, appears to be a highly polymerized and viscous form of sodium desoxyribonucleate. . . . The experimental data presented in this paper strongly suggest that nucleic acids, at least those of the desoxyribose type, possess different specificities as evidenced by the selective action of the transforming principle.

And those are the attributes of a stuff that is heterocatalytic—that can, as the gene must do, cause the cell to make another specific substance unlike itself. In support of that, Avery also observed,

> Attempts to induce transformation in suspensions of resting cells held under conditions inhibiting growth and multiplication have thus far proved unsuccessful, and it seems probable that transformation occurs only during active reproduction of the cells.

But was the transforming principle autocatalytic as well?

> Once transformation has occurred, the newly acquired characteristics are thereafter transmitted in series through innumerable transfers in artificial media [that is, repeated generations each started in fresh broth] without any further addition of the transforming agent. Moreover, from the transformed cells themselves, a substance of identical activity can be again recovered in amounts far in excess of that originally added to induce the change. It is evident, therefore, that not only is the capsular material reproduced in successive generations but that the primary factor, which controls the occurrence and specificity of capsular development, is also reduplicated in the daughter cells.

Thus Avery circumnavigated the definition of the gene. He was clear and firm about what he had demonstrated; he would not leap.

> Assuming that the sodium desoxyribonucleate and the active principle are one and the same substance, then the transformation described rep-

> resents a change that is chemically induced and specifically directed by a known chemical compound.

There was an irrepressible doubt—and the call to resolve it by a new order of scientific precision.

> One must still account on a chemical basis for the biological specificity of its action.

So the conclusion checked and stumbled:

> It is, of course, possible that the biological activity of the substance described is not an inherent property of the nucleic acid but is due to minute amounts of some other substance adsorbed to it or so intimately associated with it as to escape detection. . . . If the results of the present study . . . are confirmed, then nucleic acids must be regarded as possessing biological specificity the chemical basis of which is as yet undetermined.

That far, but in public no farther. Privately, Avery did go beyond that. A year before the results were published, he wrote a long letter to his brother, Roy, a bacteriologist then at Vanderbilt University. The letter was meditative, speculative, full of unassuming charm: it defines poignantly the sense of responsibility to science that some acknowledge. Avery first reviewed the years of searching, and then wrote:

> Try to find in that complex mixture, the active principle!! Try to isolate and chemically identify the particular substance that will by itself when brought into contact with the R cell derived from Type II cause it to elaborate Type III capsular polysaccharide, & to acquire all the aristocratic distinctions of the same specific type of cells as that from which the extract was prepared! Some job—full of headaches & heart breaks. But at last *perhaps* we have it.

He described the experimental tests, and went on:

> In short, the substance is highly reactive & . . . conforms *very* closely to the theoretical values of pure *desoxyribose nucleic acid* (thymus type) Who could have guessed it? . . .
>
> If we are right, & of course that's not yet proven, then it means that nucleic acids are *not* merely structurally important but functionally active substances in determining the biochemical activities and specific characteristics of cells—& that by means of a known chemical substance it is possible to induce *predictable* and *hereditary* changes in cells. This is something that has long been the dream of geneticists. . . . Sounds like a virus—may be a gene. But with mechanisms I am not now concerned—one step at a time. . . . Of course the problem bristles with implications. . . . It touches genetics, enzyme chemistry, cell metabolism & 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 & that evidence we are now trying to get. Its lots of fun to blow bubbles,—but it's wiser to prick them yourself before someone else tries to.

Opposition to any identification of DNA as the stuff of the gene was peculiarly concentrated at the Rockefeller Institute. Levene had been there, active until his death in 1940, the world authority on the chemistry of DNA and originator of the tetranucleotide hypothesis, by which repetitive scheme DNA could not possibly specify diversity. Alfred E. Mirsky, working in biochemical genetics there, was convinced and trying to prove that the protein associated with nucleic acids in the chromosomes of higher organisms was the active component. Mirsky argued implacably for many years, both within the institute and in public, that some proteins are resistant to the digestive enzymes used by Avery and his colleagues, so that the DNA must have been contaminated by significant traces of active protein. And every thought and argument there, not least in Avery's own lab, was shadowed by memory of a cautionary triumph of a group at the institute more than a decade earlier—the proof that enzymes are proteins and the humiliation of Richard Willstätter. In Munich, in the early twenties, Willstätter—who was perhaps the foremost organic chemist of the day, and a specialist in enzymes—had claimed that he had gotten enzymatic, catalytic action with preparations that were free of protein. On his evidence, it came to be widely accepted that the biological specificity of solutions containing enzymes was not due to protein. But in 1930, John Howard Northrop at the institute crystallized pepsin and showed that it was protein. That in itself was the second such demonstration, four years after James Sumner had done the same with urease; but Northrop and his associates developed precise techniques for correlating enzyme activity with the quantity of protein present, and showed conclusively that Willstätter's experiments had been contaminated by slight traces of protein. A laboratory colleague of Avery's for many years, René Dubos, when asked about the effect of the Willstätter scandal on Avery, replied, "It was on *everybody's* mind!"

Avery's work, even before the paper came out, was widely though unevenly known, for his laboratory had many visitors. Some papers are great, of course, because they establish, define, settle their issues. This great paper did something else: Avery opened a new space in biologists' minds—a space that his conclusions, so carefully hedged, could not at once fill up. The question was acute: If DNA is the carrier of hereditary specificity, *how*? Two scientists in particular were shocked by that question into the lines—two very different lines—their research took henceforth. Erwin Chargaff, an established biochemist then in his late thirties, on reading Avery's paper switched the work of his laboratory to the study of nucleic acids. Joshua Lederberg, just graduated from Columbia University at the age of nineteen and about to start his doctorate, found the pleasure of reading Avery's paper "excruciating"—so he noted at the time—and its implications "unlimited." He decided that these implications would never be cleared up unless bacterial inheritance could be analyzed by the methods of genetics. But bacteria were generally believed to be asexual, primitive creatures incapable of exchanging genetic information. To do genetics, Lederberg first had to show that their life cycles had a sexual stage—that they mated. On 8 July 1945, he noted down an idea for an experiment to

demonstrate genetic recombination in bacteria. The recruiting of Chargaff and the mobilization of Lederberg were among the most important effects of Avery's paper.

Avery's public caution stands in awkward contrast to the self-assurance of Watson and Crick nine years later. The cost may have been great. The Nobel Prize selectors had their attention drawn to Avery's work. They waited for the second round of discoveries. Avery was sixty-seven when the paper appeared; it was, Chargaff wrote in tribute, "the ever rarer instance of an old man making a great scientific discovery. It had not been his first. He was a quiet man; and it would have honored the world more, had it honored him more." Avery died in 1955.

I asked Crick one day about boldness and caution. "Some people of course are extremely cautious," he reflected. "Avery, exactly; he only put it in his letter to his brother. Boldness? I would have said that Bragg and Pauling were the people who most influenced me in these matters of style, and both have had that characteristic. Pauling to the point of rashness. I mean, one always knew about Linus that he would probably show an idea even if he realized, even if he knew there was a good chance of being wrong. In fact a lot of his ideas *were* wrong. But the ones that were right were important, and therefore he was forgiven for the fact that his structure of collagen was nonsense, for example, because the alpha helix and the pleated sheet were fine. But what I learned from Bragg was to grasp for the essence of the problem—and then when you've got something, get on with it and by and large publish it reasonably quickly. Though let me tell you that in the past I've often been dilatory, being from time to time of a lazy temperament. But more—from Bragg and Pauling I learned how to see problems, how not to be confused by the details, and that is a sort of boldness; and how to make oversimple hypotheses—you have to, you see, it's the only way you can proceed—and how to test them, and how to discard them without getting too enamored of them. All that is a sort of boldness. Just as important as having ideas is getting rid of them. And you realize that in those days, when we were working on DNA, the pace—things *were* very much quieter then. Now at the moment I've been thinking a lot about this problem of the chromosome structure of higher organisms, and the *rumor* of this has got *around*—the pace has got so much more hectic. I must tell you I prefer the older style—but what can one do! When we started we were living in the woods and now here we are in the middle of a city."

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At Harvard, in the early seventies, Watson several times gave an advanced undergraduate course in the biology of viruses that cause tumors in animals. One September, I went to hear his opening lectures. The course was called Biochemistry 165, and met Tuesdays and Thursdays at eleven o'clock. Watson's office was in the solid, shabby, red brick Biological Labo-