ment he said that work of the Kornberg group "in effect adds up to a handsome reward for the American people as a result of their investment in basic health research through federal agencies." Shannon went on to say, "it seems well to make this point at this time because the end products of basic biomedical research, although highly essential to progress in clinical medicine, are seldom so clearly visible in terms of potential health applications as that of Dr. Kornerg and his associates."

Now, in searching for origins of Shannon's decision to get into the picture, as well as origins of the eventual involvement of President Johnson, it is worth noting that last year the Senate Appropriations Committee paternalistically chastised NIH for failing to publicize the federal role in health research (*Science*, 28 October 1966). Last week, in an interview with *Science*, Shannon stated that this admonition was in his mind when he decided to make his statement and to bring the forthcoming public announcement of Kornberg's work to the attention of the upper echelons of the Department of Health, Education, and Welfare. When word was delivered to HEW, it was with the suggestion that the White House, which is always in quest of good news for presidential addresses, might like to take note of the event. As one person close to NIH put it, "HEW is always anxious to align the President in support of basic research. We are attempting to oppose the forces in the country that are looking for a quick return on every buck spent for basic research."

HEW conveyed news of the forthcoming report to one of the President's special assistants, and a speech writer, drawing assistance from HEW and the Office of Science and Technology, quickly drew up a few paragraphs for inclusion in the President's Smithsonian speech on 14 December. In that speech Johnson said that the work of Kornberg and his colleagues "opens a wide door to new discoveries in fighting disease and building healthier lives for mankind. It could be the first step toward the future control of certain types of cancer." He also took special note of the fact that NIH and NSF were the sources of financial support for Kornberg's research. The next day, news of the research was on front pages and TV screens throughout the country.

Associates of Kornberg said that he was astonished by the scale of public attention given the report, and that he was not a little concerned by the news media's general focus on him to the neglect of his associates. Kornberg remarked to *Science* that he thinks and hopes basic research will benefit from the publicity. He also said that, from the clippings and TV reports he has seen, he is quite impressed with the quality of scientific reporting for the general public.

And that concludes the story of how the synthesis of biologically active DNA became a major news story throughout the land.—D. S. GREENBERG

In vitro Synthesis of DNA: A Perspective on Research

2. The Scientific Aspects

To those who have followed the dramatic recent progress of research into the chemical processes of heredity, the in vitro synthesis of biologically active DNA will seem less a spectacular breakthrough than a logical extension of the discoveries of the past decade. For example, the new experiments should be viewed in the context of the analogous synthesis of infectious ribonucleic acid (RNA) in test tubes 2 years ago, by Sol Spiegelman and his co-workers at the University of Illinois. The newly reported work is nevertheless of more general interest, because RNA genomes occur in a restricted group of specialized viruses, while most known viruses and living organisms have DNA genomes.

The study of the biological synthesis of DNA at the molecular level was initiated by Kornberg with his discovery of the enzyme DNA polymerase over a decade ago. For this discovery and the work that followed he was awarded a Nobel prize in 1959. Indeed, almost everything now known about the mechanism of DNA synthesis is contained in the series of brilliant and elegant experiments published by Kornberg and his colleagues. The current report is another in the series. These papers deal with the purification and properties of the DNA polymerase as well as with the properties of the DNA produced in the enzymatic reaction. In the reaction catalyzed by the polymerase, a DNA molecule isolated from natural organisms is used as a template: four deoxyribonucleotides (adenylic, guanylic, cytidylic, and thymidylic acids) are the monomeric units which are polymerized. Polymer synthesis is such that the sequence of the four monomers in the newly synthesized chains is the complement of the sequence in the template. Complementarity is here defined by the well-known Watson-Crick nucleotide pairs.

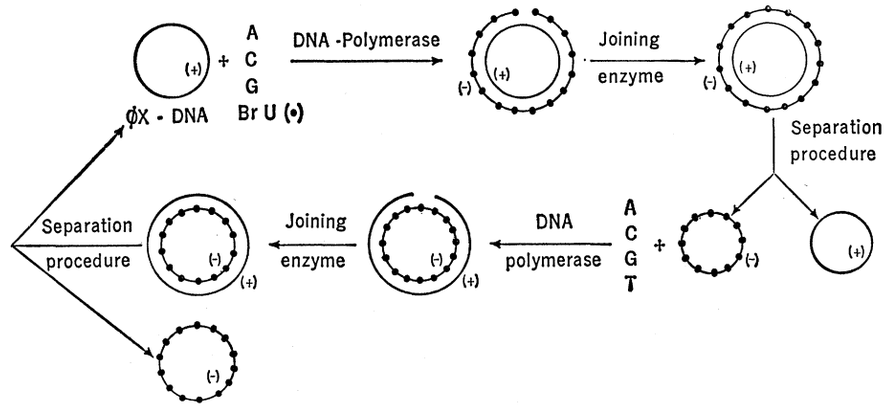
The available chemical techniques are incapable of indicating the exact fidelity of complementary copying. A DNA chain may contain 10,000 or more monomer units. One way to prove fidelity is to synthesize a DNA with a measurable biological activity—an activity which is dependent on a complete and unaltered chain. That is precisely what has now been accomplished by Goulian, Kornberg, and Sinsheimer. In these experiments, summarized in the accompanying diagram, the template copied by purified DNA polymerase was DNA from the bacterial virus ϕX174. Sinsheimer discovered this unusual virus, and it has been the center of attention in his laboratory for 10 years. The understanding of the properties of the virus and the technology developed specifically for it by the group in Pasadena were essential to the success of the latest experiment. The genome of this virus is unique: it contains 5500 nucleotide residues and only a single strand of DNA; it is not a double-stranded helical structure of the

Watson-Crick type. Furthermore, the ϕX-DNA strand is not linear, it is a covalently closed circle. This genome, stripped of the protein shell that covers it in normal, intact virus, is itself able to infect specially prepared forms of *Escherichia coli*, known as spheroplasts. Once the ϕX-DNA strand [called arbitrarily the (+) strand] has entered the cell, the cell synthesizes another strand [a (−) strand], which is the complement of the genome.

The resulting structure, called a covalent duplex circle, is a double-stranded, base-paired form, containing the (+) and (−) strands, both strands being circular. These covalent duplex circles have been isolated from infected cells, and the (+) and (−) strands were separated physically. Experiments performed in Sinsheimer's laboratory demonstrated that the isolated (−) circles are themselves infective. In these earlier experiments DNA synthesis occurred within the cell; the new experiments are significant because essentially the same events occurred outside the cell.

DNA polymerase copied the sequence of nucleotides of the (+) circle template isolated from intact virus and thereby synthesized a linear (−) structure complementary to the (+) circle over its whole circumference. However, circularity is essential for infectivity, and the chemical mechanism of the polymerization reaction does not permit the closing of the (−) linear strand to a (−) circle. Therefore the synthetic system included as an additional component a recently discovered enzyme called polynucleotide-joining enzyme. This enzyme catalyzes the covalent joining of two opposite ends of DNA chains, provided the ends are properly aligned. In this instance that alignment was assured by the hydrogen-bonding of the newly synthesized (−) linear strand to the (+) circle that served as template. The action of the joining enzyme converted the complex of synthetic linear (−) strand with the template (+) circle to a covalent duplex circle. This partly synthetic product had essentially all the properties of the natural covalent duplex circle and could be similarly manipulated to isolate separately the two component circles.

The pace of research in the biochemistry of genetics is illustrated by the fact that the first description of the joining enzyme was in a paper published by Martin Gellert, of the National Institutes of Health, in January of this year. The enzyme was almost



Synthesis of infective viral DNA. [After Goulian, Kornberg, and Sinsheimer]

simultaneously discovered in four other laboratories, several of them led by Kornberg's former students. In less than a year's time the enzyme was sufficiently well understood, and available in adequate purity, to be used for the experiments being described.

The physical separation of the two DNA circles depends in part on the fact that the new (−) circle is, by design of the investigators, denser than the template (+) circle. In the mixture of monomers used for polymerization the naturally occurring thymidylic acid was replaced by its unnatural, but biologically active, analog, 5-bromodeoxyuridylic acid. The substitution of the methyl group in thymidylic acid by the bromine atom permits a normal polymerase reaction and does not alter the infectivity of ϕX-DNA. Moreover, the increased density of the bromine-containing DNA permits its separation from the lighter template by centrifugation in a density gradient. In order to prove that the isolated (−) circles were free of contamination by the original (+) circles, the former were synthesized with radioactive phosphorus, and the latter were prepared with the easily distinguishable radioactive hydrogen (tritium). At this point, then, the investigators had obtained completely synthetic (−) circles free of natural DNA. These synthetic (−) circles were found to be capable of infecting spheroplasts, thus giving rise to a new generation of intact, normal virus. Significantly, the synthetic (−) circles were almost as efficient as infective agents as their natural counterparts are.

It is typical of the scientific elegance of Kornberg's work that the experiment just described is both confirmed and extended by other experiments published in the same paper. Thus, the completely synthetic (−) circles were also used as templates in a system exactly analogous to the one described above. This new system yielded completely synthetic covalent duplex circles, which were shown to be the same as natural covalent duplex circles with respect to various properties, including infectivity. The synthetic duplex was also separated into its component (+) and (−) circles, and finally the infectivity of the isolated synthetic (+) circle was demonstrated.

From the point of view of the specialist these experiments offer new and more rigorous proof that DNA polymerase makes few, if any, errors in copying a long chain. They also indicate that no unusual linkages occur over the 5500 nucleotides of the genome. But, as is often the case with discoveries of basic scientific meaning, they have a broader significance to science and to the humanity that science serves. They bring closer the day when the ability to manipulate genetic material can be used for improving the life of all men. The social, moral, and ethical questions that will accompany such uses were discussed by Marshall Nirenberg in a recent editorial [*Science* **157**, 633 (11 Aug. 1967)]. Quoting Salvador Luria, Nirenberg pointed out the imbalance with respect to our social framework that is created by the rapid rate of scientific progress. The scientific community has the responsibility of informing the public about progress in biochemical genetics. As concerned citizens, scientists must also take part in the general effort to assure the constructive use of that progress. We are fortunate that the scientific leaders in biochemical genetics are also men of vision who do not shrink from accepting that responsibility.

MAXINE F. SINGER
National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland