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The Effect of Annotating Articles from Scientific American on Student Understanding

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THE EFFECT OF ANNOTATING ARTICLES
FROM SCIENTIFIC AMERICAN
ON STUDENT UNDERSTANDING

by

John A. Knapp II

A Dissertation
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
Degree of Doctor of Philosophy

Western Michigan University
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John A. Knapp II
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CHAPTER I

THE PROBLEM

Introduction

An old Hindu myth suggests that the earth is supported by the "vast heads and backs" of eight male and eight female elephants, and when any one of these shakes his body, the whole earth shakes.\(^1\)

About the ground on which these elephants stand, the ancients are not clear. However, the inability to produce an airtight case does not deter man from starting to account for what he sees and thinks about.

It is difficult to justify precisely the development of new curricular materials, and state clearly the reasons for their genesis. The annotated articles prepared by the investigator and described herein, ride on the backs of at least four elephants which the investigator believes stand firm. These assumptions are:

1. The teaching of methods and processes of science is an important goal of high-school science education.

2. There is a need for developing, and a place for using, supplementary readings for high-school science.

3. Some research articles written by scientists can be read and understood by high-school science students.

4. Research articles written by scientists may be more readable and usable by means of appropriate annotations.

The Teaching of the Methods and Processes of Science

For the past several decades scientists and science educators have emphasized the importance of teaching "scientific method" in science classes in the K-12 curriculum. In 1947, Noll, in summarizing several reports on the objectives of science teaching wrote:

"The development of competence in the use of the scientific method of problem-solving and the inculcation of scientific attitudes transcend in importance other objectives in science education."¹

"There have been few points in educational discussion on which there has been greater agreement than that of the desirability of teaching the scientific method."²

Brandwein, Watson, and Blackwood³ reported an analysis (in which they did not describe the procedure) of forty-two syllabi from thirty-seven states for general science, biology, physics, chemistry, earth science, and physical science courses. The report stated that the syllabi all had one common element—the proposed goal of teaching scientific method.

In recent years major addendum to the concept of scientific method is that scientific method is not just one fixed sequence of thinking, or activity. A discussion by seventy-five scientists from several countries in a conference at Princeton University in 1947 developed the idea that "there is not scientific method as such; the


²loc. cit., p. 62.

most vital feature of the scientist's procedure has been merely to do his utmost with his mind, no holds barred."¹

Ihde² held that the scientific method is much more than a sequence of steps in problem solving and that it is dangerous to suggest that it is the frequently cited sequence. He points out instances where the sequence has been violated, or where the sequence cannot even be detected.

Currently most science educators agree that the teaching of scientific methods³, or science process, is an important goal of science education, even though the behaviors one would describe as scientific method are not necessarily fixed sequences of thought and action. However, there is much discussion concerning the optimal ways in which the behaviors that are part of scientific method can be taught. Some science educators believe that students can use science articles to learn scientific method. Later, the investigator will cite studies which support this view.


²Ihde, Aaron J., "Learning the Scientific Method through the Historical Approach." School Science and Mathematics, LIII (November 1953), 638.

³During the past four decades the name of the phenomenon called "scientific method" has changed on a number of occasions. The terms "critical thinking," "scientific thinking," "reflective thinking," "discovery method," "problem-solving skills," and "inquiry method" have been used, the most recent being "processes of science." However, an analysis of the implications of these terms indicates that they are nearly synonymous. In this study the term used will be "scientific method."
Supplementary Readings in High School Science

A major study in the value of independent readings in science was done by Curtis in 1924.¹ His conclusions based on data from secondary-science students were that (1) extensive reading adds to the pupil's achievement in general science, whether this reading is done entirely apart from any course in general science, or as an integral, or supplemental part of the regular course in general science; (2) pupils given proper encouragement and access to suitable books and magazines will read a great amount of scientific literature for recreation along with their regular schoolwork; and (3) extensive reading of scientific literature stimulates the desire of some pupils to proceed further with their study of science in school.

Because of differences in reading ability and the variety of interests of secondary students, most science educators agree that schools should provide a variety of up-to-date reading material in addition to student textbooks.

Research Articles and High-School Students

Scientific papers from professional journals often present the action, and express the excitement, of science and scientific research


*The term "research article" as used here includes that genre of article, such as found in the journals Science or Scientific American, where an investigator presents a summary of technical work on a particular problem.
better than textbooks and other condensed, or rewritten, materials.

An important study on the use of periodicals by high-school students was Baumel's doctoral dissertation, "The Effects of a Method of Teaching Secondary School Biology Which Involves the Critical Analysis of Research Papers of Scientists on Selected Science Education Objectives." Baumel maintained:

"An approach to combine the character of science with its content through the involvement of students with research papers of scientists appears to have great potential in teaching science as process. Among the many rewards to be gained from the student's first-hand contact with original writings are the genuine excitement in seeing fundamental discoveries through the eyes of their discoverer, the humanizing enrichment in becoming acquainted with the personalities of great scientists, and the possibility that youngsters will 'catch' the climate of accuracy, the careful detailed work, and the essential honesty of their scientific efforts."

In his study Baumel divided four classes of approximately thirty-five students each into two experimental and two control classes. Following the guidelines of the New York State Biology Syllabus, Baumel taught the experimental classes by having the students read and discuss certain research papers of scientists—at the rate of approximately two papers a week—whereas the control classes followed the traditional classroom activities based on a biology


textbook. Using as pre- and posttests the Cooperative Biology Test, the Watson-Glaser Critical Thinking Appraisal, and the Kuder Preference Record, the results failed to show statistically significant differences between the experimental and control groups in mastery of biology content, ability to think critically, or the expression of scientific interests. However, students in the experimental group indicated, by their responses to a questionnaire, that they found the reading and analysis of research articles beneficial in several ways. From observing the nature of the open-ended responses to several items on the questionnaire, it was evident that many students believed that their conceptions of science and scientists had changed as well as some aspects of their behavior.

In a similar study with college freshmen titled, "Effects of Teaching with Science Articles in a College, Physical Science Course," Robinson made the following assumption:

"Popular science articles differ from science textbooks not only in style, but also in purposes and in nature of contents. Consequently, articles should be superior to textbooks for accomplishing certain goals of general education in science."

In his study, 100 students in the experimental group read several articles from Scientific American instead of material from a textbook assumed to be generally parallel in content. One hundred ten students


in the control group used only a textbook. The results failed to show that there was a significant difference between the mean scores of students in the two groups on a standard biology achievement test.

To summarize, many science educators believe that teaching scientific method and the "ways of scientists" are important goals in secondary education. Further, many teachers have used science articles and research papers of scientists as primary or supplementary reading material in the classroom. In all the limited research located by the investigator, students using science articles, or research papers, have performed equally well, or better, on standard achievement measures than students using traditional textbook materials. Although reliable, generally-accepted instruments designed to measure knowledge of scientific method, or ability to use scientific methods, were not used in these studies, all researchers have concluded that science articles and research papers can be effectively used in high-school and college classes to teach scientific method.

Annotating Science Research Articles for High-School Students

The authors of many high-school science textbooks recommend articles from science journals as supplementary reading. The investigator has observed in bibliographies in current textbooks that Scientific American is by far the most frequently cited journal that contains articles considered suitable for high school students to read. In Teaching High School Science: A Book of Methods, Brandwein\(^1\) specifically mentioned Scientific American as a journal suitable for achieving

\(^1\)Brandwein, op. cit., p. 495.
certain goals in science teaching.

The investigator has examined current high-school textbooks in the area of biology, and has found that in nearly every one, with the exception of those designed for low track classes, the authors recommend certain articles from *Scientific American* as supplementary reading for expanding and developing ideas introduced. In a survey conducted by the investigator, which is part of this study, in twenty-six chemistry and four advanced science classes from eleven high schools in southwestern Michigan, 64% of the students questioned had never read a single article in *Scientific American*, and only 13% had read more than two articles. If one assumes, as do the textbook writers, that *Scientific American* contains articles that are appropriate for high-school science students, one wonders why this journal—readily available in high-school libraries and the personal libraries of science teachers—is not used more frequently by secondary students.

When students and teachers were asked by the investigator why *Scientific American* was not used more frequently, typical responses were that the articles were too difficult or too technical for beginning students to understand. In several informal discussions with students, not included as a part of this study, the investigator was told by many students that articles from *Scientific American* were too difficult to understand, had too many difficult terms, assumed too much prior knowledge about basic science, or were too boring.

On the basis of these responses, the investigator decided to select certain articles from one area of life science, namely, biochemistry, and prepare marginal annotations for these articles with an aim
at making them more readable and understandable for high-school students. This procedure will be discussed in the next chapter.

The investigator failed to locate any research that attempted to define "annotation" or attempted to measure the effects of marginally annotating materials for students to read. Consequently, as a result of this dearth of research on materials that are frequently recommended for the uses mentioned, it was decided to undertake this study.

The purpose of this study is to (1) construct a set of annotated articles from Scientific American reprints, and (2) compare, with specially constructed tests, these articles with their nonannotated analogues to determine which type of article better achieves certain objectives. The effort will be aimed at eliciting answers to these questions:

1. What is the relationship between scores on the specially designed tests when comparing the mean scores of students reading the annotated articles with those of students reading the same articles without annotation?

2. How is the amount of time reported spent reading both types of articles related to understanding the contents of the articles?

3. Do students prefer reading the annotated articles to those that are not annotated? Which students prefer which articles?
4. How is achievement in science (as measured by grades received in biology classes) related to understanding both types of articles?

5. How is achievement in English (as measured by grades received in tenth-grade English) related to understanding both types of articles?

6. How is previous experience in reading articles from Scientific American related to understanding both types of articles?
CHAPTER II

THE MATERIALS

The purpose of this chapter is to describe how (1) the articles used in this study were selected from Scientific American and annotated, (2) special tests were developed to measure student understanding of these articles, (3) a student questionnaire was developed to collect information from students who read the articles used in this study, and (4) a student-ability classification sheet was designed to identify students according to their ability.

Selection of the Articles

It was decided arbitrarily that four articles would be selected from a single discipline, namely, biochemistry. Those selected were judged to be of interest to high-school biology students, and relevant to current biochemical research by the investigator, a former high-school science teacher, and a research biochemist. (Jochanan Stenesh, a research biochemist and Professor of Chemistry at Western Michigan University, served as an advisor to the investigator and is "the biochemist" referred to in this study.) Two of the articles selected dealt with basic protein chemistry and two with nucleic acid chemistry. Their citations follow:

Having selected the articles, the next step was to identify the words, concepts, and techniques in the articles that the biochemist and the investigator judged could be clarified for beginning high-school students by definitions, illustrations, or other explanatory aids. Some examples of words, concepts, and techniques that were identified appear below:

WORDS: peptide, resin, protein, particulate.

CONCEPTS: surface-to-volume ratio, primary structure, reactive group.

TECHNIQUES: free-flow electrophoresis, paper chromatography, autoradiography.

With these elements thus identified, the investigator prepared a rough draft of definitions, illustrations, and explanatory aids. An attempt was made to make the annotation brief enough to be glossed into the margins of the pages when the 8-1/2 x 11 inch pages of the articles were mounted on 11 x 15 inch sheets of white paper. The rough draft was then reviewed by the biochemist. The annotations were then successively revised until approved by the biochemist.

Reduced photo copies of the final versions of the annotated articles appear on the following pages. The color plates and photographs,
as well as the tinted illustrations, however, appear in black and white.
THE AUTOMATIC SYNTHESIS OF PROTEINS

By anchoring an amino acid to a plastic bead one can add other amino acids one by one in automatically controlled steps. This method has already been used to make the small protein insulin.

By R. B. Merrifield

The synthesis of proteins is one of the primary functions of the living cell, and the intricate series of operations by which the cell accomplishes the task is justly known in considerable detail. Long before the biosynthesis of proteins was at all understood, at the turn of the century, the great chemist Svante Arrhenius believed proteins could be synthesized in the laboratory. It took a long time to accumulate the knowledge and techniques required to put together one of these enormously complex substances. Beginning in 1963, as the result of concerted work by many individuals, three groups of chemists—one in the U.S., one in Germany and one in China—succeeded in making in the laboratory a comparatively simple protein, the pancreatic trypsin inhibitor.

The conventional process for the chemical synthesis of proteins, or of the smaller chains of amino acids called peptides, is a slow and painstaking affair. In our laboratory at Rockefeller University we set out some years ago to look for a simpler and more efficient process—one that might lend itself to automated operation. The result was a new technique, "solid phase" peptide synthesis, in which the peptide chain is assembled not in solution but on small, solid beads of polystyrene. In 1955 the solid phase method was successfully applied to the synthesis of insulin. The availability through controlled chemical synthesis of insulin with all the properties of the natural hormone is of great value because the insulin is now used for making related molecules that differ from the parent compound in precisely known ways. Such "analogs" should help to clarify the manner in which the natural hormone functions and may in time lead to insulin derivatives that exhibit greater or more prolonged activity for the treatment of diabetes.

Even more exciting than the synthesis of insulin is the possibility of synthesizing an active one of the proteins that catalyze metabolic processes. That goal is now in sight and will surely be attained. It will add significantly to our understanding of this important class of proteins and of the mechanisms by which the living cell carries out its essential functions.

Peptide Synthesis

To understand how the chemical synthesis of a protein can be approached at all is best to look first at how peptides are synthesized. Peptides are simpler models of proteins; they consist of the same structural elements (fewer of them) linked together in the same way. The bondage between the amino acid residues is known as the peptide bond; a series of such bonds constitutes the primary "backbone" structure of peptides and proteins. The formation of these bonds is the principal problem in peptide synthesis and is also the first step in protein synthesis. In the case of proteins, however, there are also secondary and tertiary bonds that control the coiling and folding of the molecule, and these are impossible to achieve in three-dimensional space. Because the polypeptides are shorter and lack these complications, additional bonds, they are simpler to synthesize. They have been used to develop the chemistry needed to begin the synthesis work on proteins.

Amino acids are compounds containing several reactive groups; one amino group (NH2), one carboxyl group (COOH) and in many instances another reactive group located on a side chain (see page 6).

In general, all but one of these groups are protected against undesired combinations during a chemical reaction at a specific point of the molecule. The one group of one unit and the other carboxyl group of the other must be blocked. It is then possible to activate the carboxyl group of the first amino acid and to form the peptide bond.

Now let us consider extending the chain to form a longer peptide. There are two general approaches. In the "stepwise" method short peptide chains are built up and are combined to form the larger final molecule; in the "one-pot" method single amino acids are added one at a time until the final molecule is completed. (In both methods, the successive additions can in principle be made at either end of the backbone; although in practice there are certain limitations.) The stepwise technique is the older and until recently was the more common approach. Its advantages are that more of the intermediate peptides are of small size and that there are greater differences between the properties of the reactant and of the products than there are in the stepwise procedure. On the other hand, the coupling yields are generally lower in the stepwise method and there is a greater danger of unreacted side reactions.

Before any of the chain-lengthening processes can be carried out it is necessary to remove one of the blocking groups from the initial dipeptide. Topochemistry selectively in the presence

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CH₃ stands for -
NH₂ stands for -
-COOM stands for -
-OH stands for -
≡ stands for -

To the right is a list of the amino acids along with their structural formulas. Proteins, which may have molecular weights as high as millions, are made up of long chains of the amino acids which are comparatively simple in structure. These "building blocks" are used to make up the proteins of all living organisms.

To the left are some of the abbreviations used here which are standard in chemical literature.

glycine (Abbreviated "gly")
H₂N-CH₂-COOH

alanine (ala) CH₃-CO-CH₂-COOH

valine (val) CH₃-CH₂-COOH

leucine (leu) CH₃-CH₂-CH₂-CH₂-COOH

isoleucine (ile) CH₃-CH₂-CH₂-CH₂-COOH

serine (ser) HO-CH₂-CH₂-COOH

phenylalanine (phe) HO-CH₂-C₆H₅-COOH

tyrosine (tyr) HO-CH₂-CH₂-C₆H₄-OH

threonine (thr) HO-CH₂-CH₂-C₆H₄-NH₂

tryptophan (trp) HO-CH₂-CH₂-C₆H₃-NH₂

methionine (met) CH₃-S-CH₂-C₆H₄-COOH

cysteine (cys) HS-CH₂-C₆H₄-COOH

cystine (cys-cys) HOOC-CH₂-CH₂-S-S-CH₂-C₆H₄-CO

proline (pro) CH₂-CH₂-OH

hydroxyproline (hyp) HO-CH₂-CH₂-OH

aspartic acid (asp) HOOC-CH₂-C₆H₄-COOH

asparagine (asn) NH₂-CH₂-C₆H₄-COOH

glutamic acid (glu) HOOC-CH₂-C₆H₄-COOH

glutamine (gln) NH₂-CH₂-C₆H₄-COOH

histidine (his) CH₃-C₆H₃-C₆H₄-COOH

arginine (arg) NH₂-CH₂-C₆H₃-C₆H₄-COOH

lysine (lys) NH₂-CH₂-C₆H₃-C₆H₄-COOH

APPARATUS for the automatic synthesis of peptide chains is seen in the author's laboratory. It includes a small glass reaction vessel (lower right) with a -constant- "plumbing" and a programming unit (left). The rectangular piece on the rotating drum operate switches that control the pump, valves, timers and chiller that fill and empty the vessel and take the solutions. Amino acids are supplied from the six glass vessels (middle right). Solutions and other reagents are supplied from the larger containers above and at right.

page 3

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of the other protecting group (or of several such groups) and without damage to the peptide chain requires careful planning. The choice of the protecting groups and the activating, or coupling, agent for each amine and has been a major concern of peptide chemists.

At each step of the synthesis it is usually necessary to isolate, purify and characterize the products of the reaction, and it is at this point that the greatest difficulties are often encountered. Crystallization is the classical procedure for purifying peptides, as it is for many other organic compounds. It depends on the formation of an ordered array of molecules that grows to size until it precipitates from solution. Ideally each mole of one kind will be in the crystalline precipitate, and all undesired substances will remain in solution and be washed away. Sometimes one can obtain from a reaction mixture quite pure peptides that do not crystallize readily. Particularly when one is working with long peptide chains, however, the yield may often be appreciable material or crude crystalline precipitates contaminated with various by-products. One must therefore be prepared to separate these substances in addition to the desired peptide. Considerable time and effort is involved in the separation of a 100-unit polypeptide if each coupling and purification step must be performed 90 times!

The Solid Phase Approach

To synthesize molecules of the size and complexity of proteins, it seemed clear, methods of the greatest efficiency and simplicity would have to be developed...
POLYMER is a large molecule made up of repeating structural units. In the case of polystyrene, molecules of styrene (as in A below) have reacted to become repeating units of polystyrene (as in B).

Also, a protein may be considered to be a polymer made by repeatedly joining together amino acids (repeating structural units) via peptide bonds. For this reason, a protein is sometimes referred to as a polypeptide.

REACTIVE SITE: a place where there is a reactive group. (See reactive group in notes on p. 23)

CARBOXYL GROUP: the COOH or -COOH part of an amino acid molecule.

SURFACE-TO-VOLUME RATIO: refers to the numerical relationship of the surface area of an object to its volume. This is an important concept in biology on several different levels of life.

The size of many small animals is restricted in part by the high surface-to-volume ratio required by the higher metabolic activity of these animals. If an animal, or object, becomes larger and still maintains the same shape, then the surface-to-volume ratio decreases with the increase in size.

The bi-convex disc shape of the red blood cell has a very high surface-to-volume ratio. This enables the cell to exchange more oxygen and carbon dioxide than one might first expect.

The problem here in the article, however, is one of practicality and efficiency. For this process to work efficiently, the solid support must be of a size and shape to maintain a high surface-to-volume ratio.

PERMEABLE: capable of allowing matter (solid, gas, or as in this case, liquid) to pass through pores or around particles as water passes through sand.

RESIN— a three-dimensional network of atoms with many reactive sites produced by the union of a large number of molecules of one, two, or sometimes three relatively simple compounds.

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PRIMARY STRUCTURE. As used here, this refers to the linkage of amino acids via the peptide bond. The differences among proteins are due to differences in the sequence of the amino acids in the chain. (See secondary and tertiary bonds.)

The amino acid is a compound with an N-at a carbon and a carboxyl COOH of two unites with the elimination of a water molecule. Amino acids are assembled into a sequence that is specific for each protein. The primary structure of a protein is that of a chain of amino acids linked together by peptide bonds. These structures are shown in the indicated bonds. The primary structure is the fundamental feature that distinguishes one protein from another.
If the diameter doubles, then the radius would also double. Therefore, according to the formula for the volume of a sphere \( V = \frac{4}{3}\pi r^3 \), if \( r \) doubles, then, since the other variables are constant, \( V \) would become eight times as large.

Y years earlier for use in conventional synthesis, it was sensitive to certain inorganic acids that would not affect the ester, and it could therefore be removed without disturbing the aziridinyl link.

The principal remaining problem was the peptide-forming reaction itself, which had in the rapid and must not give rise to side reactions. Most important of all, it had to go to completion; it was absolutely crucial to the success of the process that this step give essentially 100 percent yields. Suppose, for example, the second amino acid were to couple to the extent of only 50 percent, which is considered a most acceptable yield in organic chemistry. What will happen then? The amino acid is coupled; it will react with the amino end of the dipeptide to form a tripeptide, but it will also couple with the unreacted 10 percent of single amino acids to produce dipeptides that lack the second amino acid. In an ordinary synthesis the intermediate products are isolated at each stage; in the solid-phase method they are simply washed free of solubles impurities, and the amino chain will be carried through the entire synthesis, giving at the end a mixture consisting of 90 percent of the correct peptide and 10 percent of a peptide with a missing link. In this sense one the two products could probably be separated and purified, but incomplete reactions were to occur several times during the synthesis of a long peptide, a complex mixture would result that might not be so easy to separate (see upper illustration on page 8).

We therefore put great stress on finding reagents and conditions that would lead to complete coupling reactions, and we have fortunately been able to achieve that goal in practice. Many ways to activate amino acids have been developed for use in peptide synthesis. For the solid phase method the most successful procedures have been to activate with the reagent diisocyanates/urethane/imidoesters or to use the nitrophenyl esters of the amino acids. These activated forms are highly effective, but only if they can reach the proper sites. To ensure their rapid, unimpeded penetration into the resin, where most of the peptide chains are located, a solvent of high swelling capacity, such as methylene chloride, is necessary.

This solvent ensures the beads to swell to approximately twice their original diameter, which means that the polymer chains are then distributed in eight times the initial volume. The polymer molecules occupy only about 12 percent of the total space of each swollen bead, and the remaining 88 percent is filled with solvent containing the activated amino acid molecules. The diffusion of these small molecules is relatively little inhibited by the polymer, so that the reactions take place almost as fast as they would in solution.

The efficiency of coupling also depends on the concentration of the reagents; the peptide chain and the amino acid being added to it. If one begins with equal amounts of the reactants, their concentrations will decrease to very low levels as the reaction nears completion.

SOLID PHASE METHOD is carried out stepwise from the carbonyl end toward the amino end of the peptide. An aromatic ring of the polyethylene (3) is activated by anilinomethyl group (2). The first amino acid (8) is attached protected by a 4-isocyanatobenzyl (I) group (black box), capped at the end (2) by a benzyl ester and is then deprotected (4). Subsequent amino acid units are supplied in one of two activated forms: a second unit is shown in one of these forms, the nitrophenyl ester of the amino acid (1). The ester (1) is eliminated as the first unit is coupled to the resin. Then the second unit is deprotected, leaving a dipeptide.
In a given chemical reaction the increase of one of the reactants causes the reaction to take place faster. For example, in the reaction below

\[ 50g \, X + 50g \, Y \rightarrow 100g \, XY \]

\( XY \) will be produced at a fixed rate depending upon the conditions. If the amount of \( Y \) is increased to 100g or 200g, then the 100g of \( XY \) will be produced at a faster rate. This happens because more collisions are able to take place between the \( X \) and \( Y \) atoms.

and the reaction rate, which is proportional to the product of the concentrations, will gradually approach zero. The practical consequence is that some of the amino acids never do link up and the reaction never quite goes to completion. This is the usual situation in conventional syntheses.

If, on the other hand, there is a rather large excess of one of the reactants, a significant rate can be maintained until essentially all the limiting reactant (the peptide chain) has entered into bond formation. To illustrate, suppose we begin with 100 parts of each reactant and call the initial reaction rate 10,000 (100 x 100). After the reaction is 50 percent completed, one part of each reactant would remain, and the relative rate would be only 1, or 1/10,000 as fast as at the beginning. Even with a very fast initial rate it would take a long time to complete the reaction.

Suppose instead we begin with 100 parts of the peptide chain as before but with 400 parts of the activated amino acid. Now after 99 percent of the chain is used up the relative rate would still be 201 (1 x 201). In the presence of the tenfold excess of the amino acid, one can calculate, the reaction can go to 99.99 percent of completion in the same time it would take to go to only 75 percent if equal amounts of the reactants had been used. One of the important advantages of solid phase peptide synthesis is that such an excess of the amino acid derivative can be used without complicating the subsequent purification procedures, since at the end of each reaction the exces is simply removed by filtration and washing. Thus we can force the reactions to completion and leave essentially no free, unreacted peptide chains.

HIGH YIELD from each coupling reaction is important in the solid phase method because products of incomplete reactions persist through the following steps. If each amino acid were to couple with even a relatively high efficiency, say 90 percent, the yield of pure peptide chain would be down to 72.9 percent by the time the fourth unit was added. More importantly, the very different peptide fragments that lack one amino acid unit or more would have to be separated chemically. In practice, yields are close to 100 percent.

**Bradykinin**, a small peptide hormone, was one of the first peptides synthesized in the author's laboratory by the solid phase method. In two amino acid synthetic (five different amino acids) were assembled stepwise from the N-terminal (right) end of the chain.
The deprotection and coupling reactions just described can be repeated alternately until the desired peptide chain has been assembled on the resin bead. The final step is the cleavage of the bulky ester bond that has been holding the chain to the resin throughout the synthesis. As indicated earlier, this bond was chosen because it is stable during the synthesis but can be selectively split at the right time, without damaging the peptide chain. The resin is suspended in methylene chloride, and dry hydrogen bromide-gas is bubbled through to effect the splitting. (More recently, anhydrous hydrofluoric acid has been successfully employed for this step.) These reactions also remove the protecting groups on side-chains. The peptide, now in a free and soluble state, is separated from the resin support by filtration and is purified. It is then ready for analysis and, where possible, for biological assay.

Those who are familiar with the mechanism of protein synthesis in the living cell will see some superficial resemblance between it and the system just described. Both depend on a separate pool of amino acids in the cell; both involve activation of the amino acids; and the cell's amino acid is activated by the energy-rich nucleotide adenosine triphosphate, or ATP. ATP and both are stepwise processes. This, however, has been learned recently that in bacteria the synthesis starts with one end of the chain protected and that an enzyme later carries out a deprotection step just as we do in the laboratory. See "How Proteins Start," by Brian F. C. Clark and Kyld A. Marcher, Scientific American (January).

The analogy should not be pressed too far, however. The natural synthesis is much more elegant and efficient than the laboratory one, and no one is sure how or even approach the complexities of the cell's scheme at this point. As a matter of fact, the chemically synthesized was not even patterned after nature; it was only in retrospect that the similarities became evident. Nevertheless, it could well be that in the future organic chemists may benefit from a more complete understanding of the way in which living systems perform their tasks.

An Automatic System

It was clear to us from the outset that an automatic mechanized process was needed for making large peptides, and that the solid phase approach would be well suited to such a process. It is an

BEADS OF POLYSYRINE, on which amino acid subunits are assembled on the solid phase method to make a peptide chain, are enclosed in 100 millimeter, 1 centimeter diameter, 1.12-cubic inch containers about twice as large when swollen as a screwdriver. Many million peptide chains can be formed on a single bead.

because the intermediate products in the synthesis need not be isolated but can be purged by simple filtration and washing reactions that can be carried out in the same vessel. The manipulations required to transfer products from one container to another have been eliminated. Once the resin beads with an amino acid attached are placed in the vessel it is only necessary to introduce the appropriate liquid solvent or reagent, allow it to react, remove the excess reagent and by-products by filtration and then repeat the process with the next reagent.

It is easy to visualize how all these steps can be accomplished automatically by a rather simple device. We constructed a machine that consists essentially of two parts: a reaction vessel with the plumbing necessary to introduce and remove the solvents in the right order at the right times and a programmer that controls these operations. The solvents, and the reagents, contained in a series of reservoirs, are selected one at a time by a spectrally designed rotary valve. The solvents are introduced into the bottom of the reaction vessel by a metering pump while air is displaced at the top. Valves in the vessel are then closed and a mechanical shaker mixes the reactants for a predetermined time. Next a vacuum withdraws solvent through a porous glass filter disk in the bottom of the vessel while dry air enters at the top, the beads, with the peptide attached, remain in the vessel. One cycle of the synthesis of the lengthening of the peptide chain by one amino acid requires 12 different reagents, one of which is a protected amino acid. The next cycle calls for the same series of reagents except for a different amino acid, which is selected by a second rotary valve.

All the steps just described are controlled by a "shaping-drum" programmer. It is like an old fashioned movie. Once the pins have been positioned on the drum to play the proper time the machine takes over and does the chemical synthesis. The pins activate switches that turn the pump and solenoid on and off and open and close the valves at the proper times, and in the proper sequence. One cycle of the synthesis requires 100 steps of the drum and takes about four hours, so that it is now possible to carry out automatically all the operations required for the assembly of a peptide chain at the rate of six amino acid/day.

After the details of the synthetic scheme had been worked out by the synthesis of small peptides the procedure was given a more demanding test: the preparation of the hormone heptadecapeptide, a nine-amino acid peptide with several physiological activities that served as

ASSAY — an analysis or analytical determination of the amount of a substance. This amount may be actual weight, or a measure of the "biological activity" of the substance.

For example, when assaying for insulin in a particular experiment, the biological activity (or measurable biological effect) of the insulin collected is compared against a defined standard of insulin activity. Biological activity of certain organic compounds is often of more interest than actual weight, and is often easier to measure. (See under p. 14.)

PARTICULATE — pertaining to or existing as minute separate particles.

In the case described here the poly-
syrene served as the anchor, or par-
ticulate support, for the building of the peptide chain. In the living cell, the ribosome similarly serves as the anchor.

RIBOSOME — one of many tiny granules in the cytoplasm of living cells. At these sites a single amino acid is anchored, and a protein built through a rather complex process.

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ANTIBIOTIC— an organic substance produced by a microorganism which has been found to be markedly inhibitory to the growth or activity of a second microorganism.

IMMUNOLOGICAL DETERMINANTS—portions of substances known as antigens which are of interest in the field of immunology. Immunology is a branch of medical science which deals with immunity (or protection from disease).

Sensitive criteria to demonstrate the identity and purity of the final product. The synthetic heptadecapeptide was identical in all respects, both chemically and biologically, with the natural hormone. During the past few years, the solid phase technique has been applied to the preparation of heptadecapeptide analogues (more than 100 of which have been made by J. M. Stuart at Rockefeller University and other small peptide hormones such as angiotensin and oxytocin. It has also been used in our laboratory and others for the synthesis of the antibodies to human albumin and tryptophan, and for synthetic studies of the immunological determinants of homologous and heterologous antigens. It was also noted that peptides containing 10 or 20 amino acids could be made in the solid phase method as well as in classical procedures. The important

First synthesis of insulin chains was accomplished by the fragment method, as illustrated here. The primary structure of the two chains is diagrammed at the center, together with the names of the amino acid units (see key at top right). Note the disulfide S-S bonds between cystine units (two of which link the A chain and B fragment chains). The general pattern of the synthesis of the

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The smallest molecule that qualifies as a true protein is insulin. It naturally became the object of intensive synthetic work by several groups of chemists when, in the late 1950s, it seemed likely that the synthesis of a protein was a feasible goal. Insulin was chosen not only for its size but also for several other important reasons. The availability of synthetic hormone would help in answering many questions about its mechanism of action. Most important, the composition and complete primary structure (i.e. sequence of amino acid units) had become known a few years before through the work of the group led by Frederick Sanger at the University of Cambridge (see "The Insulin Molecule," by E. O. P. Thompson, Scientific American, May 1955).

The insulin molecule is much more complex than a simple peptide such as bradykinin (see illustration on this page).

In their synthesis of insulin, the Sanger laboratory used the following methods:

1. The peptide bonds were synthesized in two parts, an A chain and a B chain.
2. The A and B chains were subsequently joined using disulfide bonds. The process of forming disulfide bonds was crucial to the success of the synthesis.

Disulfide bonding by these two methods accounts for much of the changing of linear peptides into complex protein molecules.
been worked out in detail, it is clear from the fact that insulin forms characteristic crystals that it is composed of molecules with a precise structure.

How can one hope to build up the long peptide chains, to form the three distinctive units between the correct cysteine units and then to fold the entire assembly into its proper shape? This is a big task, because there are many possible ways for the S-S bonds to form, and the possible variations in the conformation of the molecule are enormous. It is only possible at this time because nature comes to the chemist's aid. If we simply make the two chains with the six cysteine units in the reduced (SH) form (in which a hydrogen atom is attached to each sulfur atom) and mix them under the proper oxidizing conditions, they will preferentially form the correct S-S bridges and fold into the characteristic animal conformation all by themselves.

The discovery in 1982 by C. H. Dodds and A. C. Warshaw of the University of Toronto, that this would happen provided the key peptide chemists needed to undertake the synthesis of insulin. All four laboratories that have made insulin have depended on this fact and have made the two chains separately. From the point of view of the chemist this is a straightforward peptide synthesis rather than protein synthesis, but when the two chains are combined, the final product meets all the usual criteria for a pure compound and justifies the conclusion that a real protein has been synthesized in the laboratory.

The first published synthesis of an individual insulin chain was made by F. G. Katsuyan and his colleagues at the University of Pittsburgh School of Medicine in 1963. They made the 21-residue A chain of sheep insulin by the fragment method. When the synthetic A chain was linked with the natural B chain, the combination gave rise to a small but definite amount of insulin activity. Later that same year a large group of chemists at the Technische Hochschule at Aachen in Germany, under the direction of Helmut Zahn, reported the synthesis of both the A and the B chain, and the successful combination of the two for the first total synthesis of insulin. Over 50 percent for the A chain and 7 percent for the B chain and the extent of the combination 2.5 to 1 percent were still low, but this insulin activity was obtained. These syntheses, which also followed the fragment approach, required 39 reaction steps for the A chain and 32 more steps for the combination of the two. Each step, of source required numerous operations.

During the same period a third group was working on insulin at the Academy of Science in Shanghai and the University of Hong Kong. Their first important contribution to the problem was the development of improved methods for the separation and recrystallization of natural insulin chains. Their yield was eventually increased to about 90 percent, which meant that the combination was far from a random process. Their major contribution was the preparation in 1965 of the first crystalline, full sequence model. The crystals were obtained in low yield, but they had the same form as the native molecule and, most important, the full biological activity (more than 20 units per milligram). This was a crucial element of the proof that insulin had in fact been synthesized.

**Automatic Synthesis of Insulin**

There remained a vexing problem. Large numbers of chemists had to work for several years to produce two quantities of the peptide. In order to produce useful amounts of insulin and to be able to make modifications in the structure in a more efficient way, we undertook in 1969 to apply the solid phase method to the task. The results have been very encouraging. Although more than 5,000 separate operations were required to assemble the 51 atoms into the two chains of human insulin, most of these were performed automatically under the control of the drum programmer so that it was possible to run more than 100 sets of the synthesis of both chains in each week.

Beginning with three grams of resin, Arnold Margulis of the University of California was able to prepare approximately two grams of protected A and B chain. A total of eight grams of the protected B chain was made on eight grams of the resin. The reaction that detached the peptide chains from their polystyrene support also removed most of the side chains protecting groups, leaving only the lowest group on the cysteine and histidine side.
chains. These could be removed by reduction with metallic sodium dissolved in liquid ammonia, a reaction that was discovered many years ago by Vincent du Vigneaud of the Cornell University Medical College and was the key to his historic syntheses of the pituitary hormones oxytocin and vasopressin. Applied to insulin, however, the sodium treatment at first broke some of the bonds between the amino acids, then rehydrated and protein in the B chain. Once we recognized what was happening, it was possible to keep the chains from splitting by careful modification of the conditions of the reaction.

This deprotecting step left the cysteine groups in the reduced (SH) form. Although it was intended that the disulfide linkage should be formed during this step, the SH groups were too unstable to undergo the purification procedures that were necessary; they were stabilized by conversion to N-succinylsulfanilic acid (S-S-S). Then the two peptide chains could be purified by three methods: filtration, which depends on molecular size; countercurrent distribution, which depends on differential solubility, and free-flow electrophoresis, which depends on electric charge. The resulting products could be shown to be homogeneous by other electrophoretic and chromatographic criteria. Amino acid analysis showed that the chains had the composition characteristic of the A and B chains of insulin. The final step in the synthesis of insulin was the combination of the two purified chains. First the cystine sulfonates were converted back to the SH form. Then the chains were combined by the method developed by du Vigneaud, which involves the slow oxidation by air of the SH forms of both chains at the side chain (horizontal form), and then coupled stepwise. When the chain is complete, it is cleaved from the load, and most protecting groups are removed, by hydrogen bromide treatment.

COUNTERCURRENT DISTRIBUTION—a complex method for the separation of different proteins based upon their different solubilities in two immiscible (non-mixable) solvents.

FREE-FLOW ELECTROPHORESIS—a method of separation based upon the fact that different proteins have different electric charges in solution (due to the formation of ions). Hence proteins move to either the positive or negative electrode at rates which depend on the magnitude of the charge.

HOMOGENEOUS—of the same nature or kind.

CHROMATOGRAPHIC—the adjective for chromatography. Chromatography is a process whereby proteins and other compounds can be identified by the rate at which they move across a paper dipped in an organic solvent.
SEMISYNTHETIC — partly synthetic, partly natural. The insulin referred to is semi-synthetic because of the two chains of the molecule, one made artificially and the other obtained from natural insulin.

MOBILITY — the rate at which a material, in this case synthetic insulin, moves across a paper which has been treated with solvent. (See note on electrophoresis on p. 13.)

MYOGLOBIN — a protein molecule in muscle tissue which is similar to hemoglobin and can store oxygen like hemoglobin.

CYTOCHROME C — an iron-containing protein present in all living cells which is active in metabolism.

FERREDOXIN — an iron-containing protein which is an electron carrier in the reactions of photosynthesis.

GROWTH HORMONE — a hormone which regulates growth.

VIRUS COAT PROTEIN — Viruses are made of protein and nucleic acids. The protein covers ("coats") the nucleic acid and so is referred to as a "coat protein."

TOBACCO MOSAIC VIRUS — a virus that infects tobacco plants. It consists of a nucleic acid and some 2000 coat proteins.

CATALYST — a molecule, (in living organisms usually an enzyme), which speeds up a particular reaction without being permanently changed itself.

"SPECIFIC"— A particular enzyme catalyzes one particular reaction, therefore, an enzyme is considered "specific" for that reaction. In some cases an enzyme may catalyze several reactions, but most biochemical reactions have their own specially designed enzymes to do the job.

"ACTIVE CENTERS" — the sites on the enzyme molecules that are involved in catalytic reactions.

"SEQUENCE" — in this case, the order in which the amino acids are linked together.

Semi-synthetic insulin was partly synthetic, partly natural. The insulin referred to is semi-synthetic because of the two chains of the molecule, one made artificially and the other obtained from natural insulin.

The yield and purity of the chains themselves, which constitute the peptide-synthesis portion of the work, are quite good, but our combination yields are still poor. Important progress in that regard has been made recently by Kato et al., and their results have been published in the recent literature.

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The Author

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Bibliography


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PALEOBIOCHEMISTRY
by PHILIP H. ABELSON

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With Explanatory Notes by
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Until recently it was believed that ancient bones and shells contained none of their original organic substance. Now amino acids have been found in fossils as old as 300 million years.

PHILIP H. ALESEB

May has speculated on the origin of life at least since the Book of Genesis was first written. Today we can clearly trace the evolution of life back to the beginning of the Cambrian Period some 500 million years ago. In the Cambrian Period animals began to make hard organic substance such as shell and bone. From these hard parts we can reconstruct the outlines of soft parts—the lefs of the animals.

Until recently it was thought that the hard parts could tell us little or nothing about the chemistry of extinct organisms. The biochemical approach was largely limited to the study of living organisms, especially those which appear to differ little from their fossil ancestors. (One such organism is Limulus, the horseshoe crab.) Now, however, it has been shown that the hard parts of many ancient creatures contain appreciable amounts of their original organic substance.

In the Geophysical Laboratory of the Carnegie Institution of Washington we have recently discovered organic material in fossils as old as 300 million years. Consider, for example, a vertebra of Stegosaurus stenops, a dinosaur which lived 150 million years ago. Suppose we take a bit of this bone material and dissolve it in hydrochloric acid. In the resulting solution, we will find small amounts of various amino acids—the "building blocks" of living protein. These amino acids are glycine, alanine, proline, serine, glutamic acid, and glutamine. We will also find lower amounts of tryptophan and tyrosine.

We have found a similar assortment of amino acids in fossils from more recent formations (see table on page 6). We have also identified one, which turns out to contain no amino acids at all. Some of the remains observed from these formations which are more than 100 million years old, it seems, have been altered by high temperatures, which would cause amino acids to break down. The crystal structure of others had been replaced by new structures. For example, shells which had originally contained the lime-carbonate minerals were found to contain more normal calcite. That is because crystals which had been altered had been replaced at 2.5 degrees, and the crystals of the more nearly normal calcite. That is because of the heat of the surrounding water. These after the fashion of the calcium carbonate in their normal forms.

There is not much to be gained from a fossil of a clam shell. At the left is the shell of a modern clam of the same species. Both the fossil and the shell is shown in the diagram on the facing page.
comparison of the amounts of amino acids found in various foods, the conditions under which they were preserved vary too widely. It is of considerable interest, however, that a plate from the armor of the Devonian fish, Dunkleosteus, is extraordinarily rich in amino acids. This specimen was found in the Black Shale of Ohio, which contains very little oxygen. Thus its amino acids were shielded against oxidation.

In order to understand the origin of the fossil amino acids, we reviewed the protein content of shells and bones from living animals. It is well known that bone contains considerable protein, in some cases protein accounts for half the weight of bone. It is less widely known that protein is also found in shells. We have investigated a wide variety of shells, and have found that they always contain protein. The shells of mollusks—clams, oysters, and nautilus—have a protein content of 1 per cent to 5 per cent or more. In our experience only one of the hard substances made by living things does not contain protein. This is the sili
car skeleton of diatoms.

Although living shells contain much less protein than living bones do, fossil shells and fossil bones yield about the same amount of amino acid. This is be
cause the protein of shells often occurs in laminated sheets, so that the inner laminations are protected from attack by bacteria. Bones are not as well protected against bacterial attack. Under special conditions, however, a substantial fraction of the original organic substance in bone may be preserved. Bones taken from the lancelet La Brea tar pit in Los

PAPER CHROMATOGRAMS—papers that have absorbed dyes and various compounds that show the results of experiments in paper chromatography. This important research technique is discussed in the notes and picture captions on page 4.

DEVOHIAN—geologic time period of the Paleozoic Era (see time charts on pages 6 and 6a).

PROTEINS—a class of large complex molecules composed of amino acids. In living organisms, proteins have many functions as tissue builders, enzymes, and energy sources.

SILICA—material composed of the elements silicon and oxygen in the ratio of one to two ($SiO_2$).
ION-EXCHANGE RESIN— a man-made material, usually insoluble in water, of which some of the atoms carry a positive or negative charge. These ions will therefore attract and hold on to ions of opposite charge which are present in a solution that is treated with an ion-exchange resin.

In the present example, the positively charged amino groups (−NH₄⁺) bind to negatively charged sites on the ion-exchange resin and are in this manner separated from the other shell materials. When excess ammonia is added, the ammonium ions (NH₄⁺) replace the amino acids on the ion-exchange column. The amino acids are removed and the ammonium ions are now bound to the resin. Because of this process such ion exchange resins are known as cation exchangers.

Ion exchange is an important method used by biochemists to separate organic compounds. Ion exchange is also used to soften tap water. In this case one also uses cation exchangers, substituting Na⁺ for Ca²⁺, Mg²⁺, and Fe³⁺ present in hard water. All ion-exchange reactions are similar in principle to the one discussed above.

\[ 2 \text{HCl} + \text{CaCO}_3 \rightarrow \text{CaCl}_2 + \text{CO}_2 + \text{H}_2\text{O} \]
PAPER CHROMATOGRAPHY—a widely used method of separating and identifying organic compounds based on the fact that these compounds will travel by adsorption at different rates along a piece of paper soaked in a solvent. The molecules being traced are stained with a dye to make them visible. By comparing chromatograms produced from an unknown mixture with chromatograms made from compounds of known composition, identification of organic molecules can be made.

PECPE CHAINS: two or more amino acids combined together in a special way to form a larger complex. This bond (between the acid part of one amino acid and the amino part of another) is called a peptide bond.

CARBON 14 METHOD—a widely used method of determining the age of carbon-containing compounds that are no more than 50,000 years old.

This technique is possible because living organisms continually take in a specific ratio of C12 and C14 atoms. Both of these are used in the building of organic compounds. Since atoms of C14 contain two extra neutrons in their nucleus, they are unstable and radioactive. These atoms “decay”—emitting radiation and breaking down into atoms of a different kind at a known rate. Hence the number of radioactive carbon atoms in a given sample decreases with time. Knowing the rate of decay of carbon 14 and the original and final percentages of carbon 14 in a sample, the age of an organic compound can be calculated.

This method is reliable in calculating the age of compounds that are less than 50,000 years old. After this time the amount of C14 that remains is too small to be accurately measured. For compounds older than this, other radioactive methods may be used. Many compounds contain the radioactive atoms X40, U235, U238, or Ti72. These atoms decay much more slowly than C14, therefore, for this and other reasons these atoms are better “clocks” for dating very old compounds.
rapidly than the others. After a few million years they will vanish altogether. This explains why various fossil flakes contain the same unusual assortment of amino acids. Originally they all consisted of proteins composed of the same amino acids we find in proteins today, but only the stable amino acids have survived.

What about the possibility that the amino acids we extract from fossils do not correlate with the original protein of the material, but with modern sources? Are our specimens, in short, merely contaminated? It would be difficult to eliminate this possibility if we considered only one fossil. The fact that we find similar amino acids in fossils of the same species collected from many different formations strongly supports the assumption that the same amino acids in the fossils were those during the life of the animal.

Another indication that we are dealing with the original material has to do with the way in which certain amino acids are adsorbed on calcium carbonate precipitating in the laboratory. Aspartic acid and glutamic acid tend to be adsorbed on such precipitates; other amino acids are not. The fossil material is largely composed of calcium carbonate, thus if the amino acids in it had been adsorbed from water in the ground, we would expect to find an abundance of aspartic acid and glutamic acid. Such is not the case.

We must also consider another point. Are amino acids sufficiently stable to last for long periods of geologic time? Fixed with such a question, the average chemist would probably say no. Fortunately, the question may be answered by referring to an approach used in test products. In this approach tests are designed to subject the product to the equivalent of many years of service within a few hours or days.

In the case of amino acids the test consists of heating these compounds to high temperatures and observing their rate of breakdown. In this way we were able to study the breakdown of alanine in some detail. The kind of reaction in which alanine breaks down proceeds at a rate which is described by a well-known law of physical chemistry: the Arrhenius equation, named for the famous Swedish chemist Svante Arrhenius. By observing the behavior of alanine at various high temperatures, it is possible to draw a curve which can be extended to predict its behavior at relatively low temperatures. We found that at 450 degrees centigrade it took about a second for a given concentration of alanine in water to decrease by 55 per cent. At 185 degrees C. it took a month for the concentration to decrease by the same amount. If we extend the curve based on such figures, we find that at room temperature alanine could last for billions of years. Many other organic compounds are even more stable.

We have similarly accelerated the aging of other amino acids, though not in so much detail. These studies have shown that the most stable amino acids are alanine, glutamic acid, glycine, isoleucine, proline and valine. The less stable amino acids are arginine, aspartic acid, histidine, phenylalanine, serine, threonine, and tyrosine. Thus our laboratories studies correlate perfectly with our findings in fossils.

When we investigated the breakdown of phenylalanine we encountered an interesting possibility. Phenylalanine decomposes into carbon dioxide and phenethylamine, which is much more stable than the compound from which it is derived. Phenethylamine does not normally occur in living organisms. If we were able to isolate it from a fossil, we would have good ground for the assumption that the fossil had once contained phenylalanine. When we learn more about the decomposition of other amino acids we shall have additional evidence in support of the hypothesis that certain amino acids are present in fossils as evidence of life of long standing.

**ARRHENIUS EQUATION**—a mathematical expression which relates reaction rate to temperature.

As a rule of thumb, with all other variables being held constant, it has been observed that the rate of a chemical reaction doubles for every ten degree rise in temperature. Svante Arrhenius, a Swedish chemist, developed through experimentation an equation which shows the relationship between reaction rate and temperature.
Below is a chart of geologic history which has been adapted from Eicher, Geologic Time, Langewell, Introduction to Physical Geology, and Namowitz, Earth Science. This chart helps put the geologic periods ("late Pliocene, Miocene, . . . Devonian") mentioned in the figure on the previous page into perspective.

"Geologic time" runs from the present back to about 4,500,000,000 years ago. The dates for the geologic periods have been calculated by measuring the decay of certain radioactive atoms present in rocks. How this works has been discussed in the notes on carbon-14 method on p. 5. Because there is a margin of error in using radioactive dating methods, geologic time scales can only approximate the dates of the geologic periods. This also accounts for the variation in time charts in different books.

In the chart below the development of certain animals such as the dinosaur and the elephant have been singled out to illustrate the rise and fall of certain species throughout geologic time.

<table>
<thead>
<tr>
<th>ERA</th>
<th>SYSTEM &amp; PERIOD</th>
<th>EPOCH</th>
<th>DISTINCTIVE EVENTS</th>
<th>DATES</th>
</tr>
</thead>
<tbody>
<tr>
<td>CENOZOIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TERTIARY</td>
<td></td>
<td>Recent</td>
<td>Man dominant. Domestic animals develop.</td>
<td>300,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pliocene</td>
<td>Man appears. Elephants die out in North America.</td>
<td>11,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Miocene</td>
<td>Horses and large carnivores develop. Elephants dominant.</td>
<td>2,500,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oligocene</td>
<td>Grazing animals thrive. Whales, monkeys develop. Elephants migrate to North America.</td>
<td>7,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eocene</td>
<td>Mammals progress. Elephants develop in Africa.</td>
<td>25,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paleocene</td>
<td>First horses develop. Many new mammals appear.</td>
<td>39,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>54,000,000,000</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65,000,000,000</td>
</tr>
<tr>
<td>MIOCENE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CRETACEOUS</td>
<td>Flowering plants develop. Dinosaurs die out.</td>
<td>130,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JURASSIC</td>
<td>&quot;The Age of Dinosaurs.&quot; The first birds develop.</td>
<td>190,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TRIASSIC</td>
<td>First dinosaurs develop. First primitive mammals develop.</td>
<td>225,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PERMIAN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PENNSYLVANIAN</td>
<td>First reptiles appear. Great coal-forming forests. Abundant insects.</td>
<td>260,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MISSISSIPPIAN</td>
<td>Amphibians flourish. Ferns, cone-bearing trees abundant.</td>
<td>325,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DEVONIAN</td>
<td>First amphibians appear. Earliest forests.</td>
<td>345,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>395,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SILLURIAN</td>
<td>First land plants and land animals appear.</td>
<td>430,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OROVICIAN</td>
<td>Earliest primitive fishes develop.</td>
<td>500,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAMPBRIAN</td>
<td>Many marine invertebrates such as trilobites, brachiopods, snails, and sponges are abundant.</td>
<td>570,000,000,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4,500,000,000,000 (1)</td>
</tr>
</tbody>
</table>

page 6a

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**EXPERIMENTAL NUMBERS** - a shorthand way of expressing very large or very small numbers.

To illustrate:

- $10^2$ means 10 times 10, or 100.
- $10^3$ means 10 times 10 times 10 times 10, or 10,000.
- $10^4$ means 1/10 times 1/10, or 1/100, or .01.

**PORPHYRINS** - a class of organic compounds having a nitrogen-containing ring system which is of extreme importance in the maintenance of both plant and animal life. These compounds are usually brightly colored, and combine readily with metal ions. Chlorophyll and hemoglobin contain porphyrins. The "heme" portion of hemoglobin is shown at the right.

**HYDROCARBONS** - compounds which consist solely of the elements carbon and hydrogen.

**CAROTENOIDs** - a class of pigments which vary in color from deep red to yellow that occur in plant and animal material.

- *Carotene*, one of the carotenoids, may be written as C40H56, or as illustrated at A at the bottom of the page.

**ALCOHOLS** - a class of hydroxylic (OH) containing compounds, the simplest of which has the general formula CnH2n+1 OH. For example, butanol, one of the alcohols, has the formula C4H9OH or CH₃CH₂CH₂CH₃ OH.

**FATTY ACIDS** - organic acids which occur in natural fats and oils. An example of a fatty acid is stearic acid which may be written

C₁₈H₃₃COH or (CH₃)₂₇COH

**LIGNITE** - BROWN COAL, BITUMINOUS COAL, ANTHRACITE - various grades of coal which may be ranked in the following order to indicate increasing amounts of fixed carbon which they contain:

- **Brown coal** - about 90% fixed carbon; resembles peat.
- **Lignite** - about 70% fixed carbon, most of the rest of the weight being moisture.
- **Bituminous coal** - "soft coal"; contains about 80% fixed carbon.
- **Anthracite** - "hard coal"; contains about 95% fixed carbon.

**ALGAL** - the adjective for algae. Algae are single-celled organisms which are usually photosynthetic, that is, they are capable of taking energy from sunlight and carbon dioxide from the air to make their own food.

**ALANINE WAS HEATED** to various temperatures to determine whether it could persist for long periods. The horizontal coordinate of the small circles indicates the temperature to which each sample of alanine was heated. The vertical coordinate indicates the length of time it took for 43 per cent of the alanine in each sample to break down. If a straight line is drawn through the circles, it is apparent that at a temperature of 20 degrees centigrade nearly half of a given amount of alanine would remain after three billion years.

**ACIDS** - it may be possible to infer the existence of unstable amino acids that have broken down.

**Amino acids are of course only one of** many families of chemical compounds synthesized by living things. There is much evidence to indicate that compounds in other groups can also survive for millions of years. The group to which the **porphyrins** belong is the **porphyrinoids**. An iron porphyrin is present in the blood pigment hemoglobin; indeed, all organisms that require free oxygen contain some kind of porphyrin. Large quantities of vanadium porphyrins have been found in petroleum. In one case it was apparent that the oil had been exposed to a temperature of 150 degrees C for tens of millions of years. This survival indicates that porphyrins are far more stable than amino acids. If porphyrins were manufacture throughout the earliest forms of life, some of these substances should still be in existence.

Other constituents of petroleum provide evidence on the life of the past. Frederick D. Rossignol at the Carnegie Institute of Technology has isolated some 140 organic compounds in crude oil from the mid-continent deposits of U. S. This oil, like many others, is rich in hydrocarbon molecules which have the shape of a single chain. Now all modern organisms contain fatty acids. When fatty acids are broken down by bacteria or heat, they yield single-chain hydrocarbons. Laboratory experiments indicate that only moderate heat is required to encourage this reaction. The survival of petroleum in formations where it has been exposed to temperatures above 200 degrees C is testimony to the durability of some organic matter.

It has long been known that coal is rich in organic compounds. By means of mild chemical treatment that does not alter the original molecules, a host of such compounds has been isolated. Among them are porphyrins, carotenoids, hydrocarbons, and fats. The kind and amount of organic substance found in coal depend on its thermal history. Lipids and humus coal are richer in these compounds than bituminous coal or lignite.

Perhaps the most exciting prospect for the study of fossil organic compounds lies in the vast stretch of time before the Cambrian Period. Although estimates of the age of the earth vary and are subject to change, the earth is at least 3.5 billion years old. A wide variety of living organisms existed at the beginning of the Cambrian Period some 500 million years ago. What forms of life existed during the preceding period? What was the chemistry of these organisms? When, indeed, did life originate?

These questions are difficult to answer because of vast changes that have occurred in the earth's crust since Pre-Cambrian time. The sediments that settled in Pre-Cambrian seas have been deeply buried and often folded. The high temperatures associated with these processes might obliterate the survival of some organic substances. The absence of fossil land forms further narrows the range of material that may be studied.

There are nonetheless good opportunities in the Pre-Cambrian black shale, which contains a certain amount of hydrocarbons. Small quantities of a petroleum-like substance have been observed oozing from rock tiles in Michigan. Pre-Cambrian shales in Sweden and Finland are relatively rich in organic matter. Else B. Johnsen of Harvard University has recently extracted an organic pigment from a black shale with an estimated age of 1.4 billion years. Similar studies will doubtless reveal a large number of organic compounds in Pre-Cambrian rocks. It is not impossible that the soft parts of Pre-Cambrian organisms have been preserved. One possibility of preservation may be observed today near hot springs. As the water draws away from the orifice of the spring, it cools and makes an excellent culture medium for algae. These water is saturated with silica, which precipitates and traps the algae. Thus the organisms are sealed in rock, an ideal medium for the preservation of organic matter. Barrois and Stanley A. Tyler of the University of Wisconsin have reported the discovery of fossil structures embedded in almost pure Pre-Cambrian silica.
The Author

PHILIP H. ABBEY is Director of the Geophysical Laboratory of the Carnegie Institution of Washington. He was born in Tacoma, Wash., and graduated from the State College of Washington in 1933. He started out as a nuclear physicist, doing graduate work with Ernest O. Lawrence at the University of California. He took his Ph.D. there in 1939, in 1940 he and several other workers discovered the synthetic element neptunium. At the Naval Research Laboratory during World War II he developed a liquid thermal diffusion process for separating uranium isotopes on a large scale; this process was later used at Oak Ridge. In 1946 he began a new career in biochemistry and microbiology at the Department of Terrestrial Magnetism of the Carnegie Institution and acquired an interest in comparative biochemistry, evolution, and the origin of life.

Bibliography


ANNOTATORS

JOHN KNAPP II, a former Peace Corps volunteer and high school chemistry and general science teacher, graduated from Wheaton College in 1963 and earned an M.A.T. in general science from Michigan State University in 1968. He is presently completing his Ph.D. in science education at Western Michigan University in Kalamazoo, Michigan.

JOCHANAN STENESCH obtained his Ph.D. degree in biochemistry in 1958 from the University of California at Berkeley. He is professor of chemistry at Western Michigan University where he is actively engaged in research on the molecular biology of proteins and nucleic acids.
INTRODUCTORY NOTE

In a beginning study of biology we soon learn that all living things have many things in common. All organisms require certain materials—foods, gases, etc. All organisms give off waste products. All move to some extent by their own power. All reproduce their own kind. All "die" and break down into simpler materials that do not have these properties.

DIFFERENCES AMONG ORGANISMS

One of the interesting mysteries about living things is that they are different. A protozoan does not look like a horse, though both perform similar chemical activities to stay "alive." If we look at animals that more closely resemble each other, say dogs, we still find even here a multitude of organisms of every size and shape which are capable of mating and producing offspring which may look different than either parent.

Even among humans we see great differences in size, weight, skin color, and intelligence. Human children in many ways resemble their parents, yet often there are striking contrasts between a child and his parents, or between brothers and sisters.

Looking at the record of fossils in rocks, we also see changes in organisms with the passage of time. In general, living things seem to have become more complex as time passed.

What accounts for the changes in organisms? What accounts for the variety among individuals of the same kind? Why are not all people alike? carbon copies of each other? Why isn't a child built exactly like his parents? How does a single cell change into a baby? A baby into a man?

Scientists who study genetics and biochemistry are seeking answers to these questions. Today, we know that a very complicated series of chemical reactions brings about these changes. Further, we know that the single cell (which eventually becomes an adult individual) has all the information that is necessary to bring about these chemical reactions.

We have discovered that this information is found in complex molecules in the nucleus of the cell. Only within the past twenty years has it been shown that long helical (twisted "ladder-shaped") molecules named DNA (deoxyribonucleic acid) are responsible for directing the activities of the cell.

Since the discovery of DNA scientists have been hard at work trying to determine more clearly its structure and function. Today, it is believed that although the DNA of every living organism is made up of similar subunits, that THE PRECISE WAY THESE SUBUNITS ARE CONNECTED TOGETHER IS RESPONSIBLE FOR THE DIFFERENCES OF INDIVIDUAL LIVING ORGANISMS.

To sum up, an individual receives his own unique DNA when he is born. This DNA contains the "blueprints" of the individual. The DNA molecules also act as the "boss" of all molecular construction within an organism.

Of particular interest to scientists is the discovering of just how the DNA in a particular cell goes about its building activity. The Genetic Code: II by Marshall W. Nirenberg deals largely with this question.

Why go to so much trouble to understand the genetic machinery in the cell? If scientists can pin down just how DNA works, and learn how to recognize certain types of DNA, then we are one step closer to preventing or curing certain types of hereditary disease. Then, too, there is the satisfaction of discovering the unknown. Behind every piece of real research is a curious, questioning mind.
THE GENETIC CODE: II

A sequel to F. H. C. Crick’s article of last October, which discussed how the hereditary material embodies the code for the manufacture of proteins. The nature of the code has now been further elucidated.

by Marshall W. Nirenberg

J ust 10 years ago James D. Watson and Francis H. C. Crick proposed the now familiar model for the structure of DNA (deoxyribonucleic acid), for which they, together with M. H. F. Wilkins, received a Nobel prize last year. DNA is the giant hereditary molecule that embodies the genetic code of all living organisms. In the October 1962 issue of Scientific American, Crick described the general nature of this code.

The mechanism of the code is this: Each gene directs the manufacture of a specific protein. Each gene consists of a linear sequence of bases that is the basis for the synthesis of a specific protein. The code is read in groups of three bases, called codons. Each codon specifies a particular amino acid, and the sequence of codons along a gene specifies the sequence of amino acids in a protein. The genetic code is universal; it is the same for all organisms.

Four bases - adenine, guanine, cytosine, and thymine - are the four nucleotides in DNA. Each base can pair with only one other base: adenine with thymine, and guanine with cytosine. This base pairing is the basis of the double helix structure of DNA.

The genetic code is universal, as are the chemical reactions that translate it into protein. The same code is used in all organisms, from bacteria to humans. This universality is a fundamental aspect of life and a testament to the elegance of nature.

There are 64 possible codons, but only 20 amino acids, so some codons do not correspond to any amino acid and are considered "stop" or "nonsense" codons.

The genetic code is read from the 5' to the 3' end of the DNA strand, which is why DNA is said to be "univalent" or "single-stranded".

The code is degenerate, meaning that more than one codon can specify the same amino acid. This redundancy is thought to be a way of ensuring that the code can tolerate mutations.

The discovery of the genetic code is one of the greatest achievements in the history of biology. It has revolutionized the way we think about life and has opened the door to a unparalleled understanding of the molecular basis of inheritance and variation. The genetic code continues to be a subject of active research, with new discoveries and refinements being made all the time.
CELL-FREE - This means that the cell walls of certain cells have been carefully broken and all of the cytoplasm, dissolved or suspended material, has been taken out. Great care has been taken in cell-free systems to make sure conditions outside the cell are not too different from those inside the cell. If, for example, the temperature is too high, or the pH (acidity) changes too much, the cell materials in the cell-free system will break up and be destroyed.

MESSENGER RNA - See "3" in the diagram on p. 6 and the explanation of RNA in the notes on p. 4.

RADIOACTIVE - spontaneously emits radiation (or particles) from the nucleus of an atom. Some atoms do this naturally because their nuclei are unstable. Other "normal" atoms can be made radioactive by various treatments. Using compounds that have been built up containing certain specially supplied radioactive atoms, is an important research method of science. These compounds which are called "tagged" can readily be traced and measured by using geezer counters and other devices. The "tagging" of compounds does not ordinarily affect their characteristic chemical properties.

In the case described here, it is easy to check how much of an amino acid (made radioactive) has been used simply by measuring the radioactivity of the final solution.

Note the comparatively simple equipment and procedure here. Man-made RNA (which resembles DNA) is used so that the scientist knows just which bases are at work. After this RNA does its work of arranging the amino acids (with one amino acid "tagged") into a protein, this protein is precipitated out of solution. A suction filter is used to collect the protein, after which the radiation counter measures how much of the tagged amino acid has been taken up.
RNA (RIBONUCLEIC ACID) — Whereas DNA is the genetic material which directs the activities of a cell, RNA is the molecule, or molecules, that "translates" or carries the directions from the DNA to the parts of the cell where proteins are made.

This nucleic acid resembles DNA if you split the "rungs of the ladder" (or break the bonds that connect the pairs of bases). See diagram at the bottom of p. 2.

**Messenger RNA and transfer RNA** are two of the several kinds of this nucleic acid. RNA is discussed in more detail on p. 5.

**RIBOSOMES**— granules of RNA and protein in the cytoplasm of every cell, that are so small they can only be seen through an electron microscope.

Proteins are made at the ribosomes. (See diagram on p. 6.)
ADENOSINE TRIPHOSPHATE—more frequently referred to as "ATP"—is a phosphate compound found in all cells, that is produced from the breakdown of food. The key compound provides the driving energy for most chemical reactions within the cell. The structure of ATP is at the right.

Note the three phosphate groups. (A phosphate group is a phosphoric acid, or P, together with four oxygen atoms.) Because of these three phosphate groups, this compound is called "adenosine triphosphate." The brown to the MAJOR arrow is broken when ATP releases its energy. When this happens, ADP (adenosine diphosphate) is produced. Note also that ATP contains adenosine, one of the nitrogen bases (see diagram on p. 4).

COMPLEMENTARY—the adjective of complement which means "that which is needed to fill a thing or make it complete; one of two mutually accomplishing parts." (Webster's Dictionary)

If we split the ladder of DNA down the middle of its rungs, we get two portions of the molecule which are "complementary" to each other. This is so, because a particular base can only combine with another particular base (i.e. base "G" can only combine with base "C").

In the case in this article, the RNA (which resembles a molecule of DNA split down the middle) found in a cell is said to be "complementary" to one of the strands of DNA (see the diagram at the bottom of the page). Note that the bases in C are complementary to A, or are the same as A. If we substitute U for T (U is very similar to T. See diagram on p. 4).

RIBONUCLEIC ACID—As in most biochemical reactions, this is a special protein (enzyme) which catalyzes a particular reaction, or series of reactions—in this case, these reactions which build up RNA from smaller subunits.

ON THE PATTERN—As said before, DNA serves as the template, or pattern, for making RNA. We are not sure just how the RNA is made, only that it has been designed by DNA.

So, just as every individual organism has a unique pattern for its DNA, every organism has unique, complementary molecules of RNA.

COFACTOR—a small molecule which some enzymes need in order to be fully active.

COLON BACILLUS (Escherichia coli)—(commonly called E.coli). This microorganism, which occurs normally in large numbers in the intestine, has been used extensively in biochemical and genetic research.

TRANSCRIPT—makes a copy of.
progress was slow because we had to prepare fresh enzyme extracts for each experiment. Later my colleague J. Heinrich Matthaei and I found a way to stabilize the extracts so that they could be stored for many weeks without appreciable loss of activity.

Normally the proteins produced in such extracts are those specified by the cell's own DNA. If one could establish the base sequence in one of the cell's genes—or part of a gene—and correlate it with the amino acid sequence in the protein coded by that gene, one would be able to translate the genetic code. Although the amino acid sequence is known for a number of proteins, no one has yet determined the base sequence of a gene. Hence the correlation cannot be performed. The study of cell-free protein syn-

SYNTHESIS OF PROTEIN begins with the genetic code embodied in DNA (1). The code is translated into messenger RNA (2). In the diagram it is assumed that the message has been derived from the DNA strand bearing dark letters. The messenger RNA finds its way to a ribosome (3), the site of protein synthesis. Amino acids, indicated by numbered squares, are carried to proper sites on the messenger RNA by molecules of transfer RNA (see illustration on opposite page). Boxes are actually equivalent, but...
The thesis presented an indirect approach to the coding problem. Tarrant, Noll and Breitman, work at the Pasteur Institute, had reported that protein synthesis could be blocked in wheat roots by adding chloramphenicol, an RNA synthesis inhibitor that specifically inhibits synthesis of RNA. Pataki and J. M. also showed this effect and studied its characteristics. It seemed probable that protein synthesis was blocked after the messenger RNA had been degraded. When we added such factors of messenger RNA synthesis that we found that they stimulated protein synthesis. To reproduce this observation for messenger RNA derived from Escherichia coli, we examined the amino acid for an undefined role.

We obtained RNA free from an amino acid mixture, including amino acids, and found that only two of them were highly active in directing protein synthesis in the cell-free system of the cobdenlassen. These amino acids were found to accept RNA "blueprints" obtained from other organisms, including viruses. It should be emphasized that only minute amounts of proteins were synthesized in these experiments.

It occurred to us that synthetic RNA containing only two amino acids might direct the synthesis of simple proteins containing only two amino acids. Synthetic RNA molecules can be prepared with the aid of certain polyribonucleotide phosphorylase, found in 1955 by Maxamam, Gellertia, and Simon and their colleagues at the New York University School of Medicine. Unlike RNA polymerases, this enzyme does not follow the pattern of DNA. Instead it forms RNA polymers by linking bases together in random order.

A synthetic RNA polynucleotide consisting only of two nucleotides called polyadenylic acid, was prepared and added to the cell-free system, together with one of the two amino acids. For each mixture, one of the amino acids was added to the RNA in addition to the other RNA. If a mixture was added to the RNA in addition to the other RNA, the RNA was determined by the particular amino acid and directed into the protein, by polypeptide C. In this way, one could determine the amino acid in the ribosome and directed into the protein. By polypeptide C.

It proved to be the amino acid phenylalanine. This evidence indicated that the RNA codons for phenylalanine were a sequence of GUU (mixture in polypeptide C). The code word for another amino acid, alanine, was found to be GUU (polyphenylalanine, polypeptide C). Thus, a cell-free system capable of synthesizing proteins based on the direction of the mRNA could be used to direct the synthesis of RNA, by polypeptide C. A simple means for determining the genetic code.

The Cold-Weather Dictionary

[Diagram of nucleotides and amino acids]
POSSIBLE TRIPLETS—With a triplet of three nitrogen bases, and four different nitrogen bases, there are $4^3$ or $4 \times 4 \times 4 = 64$ different possible sequences.

To illustrate how probability works, suppose we want to predict, mathematically, what will happen when we toss a coin. If we flip a penny, the chance is “50-50” (or “1 out of 2”) that we will get heads. This can be expressed by the decimal .5. Suppose we want to know the chance of getting heads twice in a row. With the first toss, the chance we will get heads is .5 (as before). The same is true of the second toss. To calculate the chance of getting heads both times we multiply these two “probabilities” together:

$.5 \times .5 = .25$

(continued on next page)
Hence the probability of getting heads twice in a row is "25 out of 100" or "1 out of 4."

Similarly, if we want to predict the chance of getting heads 3 times in a row, we can calculate:

\[ 0.5 \times 0.5 \times 0.5 = 0.125 \]

which means that the probability of getting heads all three times is "125 out of 1000" or "1 out of 8."

To take another example, suppose we have a bag which contains 70 black marbles and 30 white marbles that are well mixed together. Using similar reasoning, if we blindly take one marble out of the bag the chance is "70 out of 100" or 0.7 that the marble will be black.

If we want to know the chance of picking two white marbles out of the bag in a row, we can predict this by calculating:

\[ 0.7 \times 0.7 = 0.49 \]

which means that the chance of accomplishing this is "49 out of 100" or 0.49.

Similarly, the chance that we will get two white marbles in a row is:

\[ 0.3 \times 0.3 = 0.09 \]

The chance that we will get a black marble and then a white marble is:

\[ 0.7 \times 0.3 = 0.21 \]

This kind of reasoning is used in analyzing the results obtained from experiments using synthetic RNA. In the case described in this article, synthetic RNA was made by reacting a mixture of bases yielding a product (poly-UC) which contained 70\% uracil (U) and 30\% cytosine (C). Therefore, if we assume that the sequence of bases in poly-UC is a result of random combinations, then we can predict that this synthetic RNA will be composed of eight possible triplet combinations which will occur with the frequencies given in the chart below:

<table>
<thead>
<tr>
<th>BASE SEQUENCE (8 possibilities)</th>
<th>CALCULATION OF PROBABILITY</th>
<th>% OF THIS TRIPLET PATTERN IN FINAL MIXTURE OF SYNTHETIC RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>UUU</td>
<td>.7 X .7 X .7 = .343</td>
<td>34.3%</td>
</tr>
<tr>
<td>UUC</td>
<td>.7 X .7 X .3 = .147</td>
<td>14.7%</td>
</tr>
<tr>
<td>UCC</td>
<td>.7 X .3 X .3 = .063</td>
<td>6.3%</td>
</tr>
<tr>
<td>CCC</td>
<td>.3 X .3 X .3 = .027</td>
<td>2.7%</td>
</tr>
<tr>
<td>CUC/U</td>
<td>.3 X .3 X .7 = .063</td>
<td>6.3%</td>
</tr>
<tr>
<td>CUU/U</td>
<td>.3 X .7 X .7 = .147</td>
<td>14.7%</td>
</tr>
<tr>
<td>CUC/U</td>
<td>.3 X .7 X .3 = .063</td>
<td>6.3%</td>
</tr>
<tr>
<td>UCU/U</td>
<td>.7 X .3 X .7 = .147</td>
<td>14.7%</td>
</tr>
<tr>
<td><strong>total = 1,000</strong></td>
<td></td>
<td><strong>100.0%</strong></td>
</tr>
</tbody>
</table>

After preparing synthetic RNA that has a "70:30" (uracil : cytosine) composition, we can use it to make a synthetic protein from the 20 amino acids. By analyzing the amounts of the various amino acids that have been taken up by the protein, we can suggest which particular triplet pattern brought in a particular amino acid.

Looking at this experiment theoretically (ignoring some of the practical problems), suppose that an analysis of the protein produced contained, among other amino acids, 34\% phenylalanine and 3\% proline. Then we could suggest that the triplet UUU was responsible for bringing in the phenylalanine, and CCC was responsible for bringing in proline (see chart).

By doing experiments of this type, biochemists are discovering which triplet patterns code which amino acids.

This research is complicated by the fact that amino acids may respond to more than one triplet code, just as one amino acid may respond to the same triplet code (this is very rare, but true for UUU), and that while making synthetic RNA the nitrogen bases may not randomly combine on the ladder of the molecule.

---

* The logic and calculations here, and in the following examples, is not strictly correct as the selection of one marble (or amino acid) reduces the number of marbles left in the "pool." However, for illustrative purposes we will assume the number of available marbles, and amino acids to be the same. This simplifies the calculations. Also, when the pool of choices is large, these simplified probabilities do not vary much from actual probabilities.
real, and in gene expression in protein synthesis. This would occur if nucleotides were to direct two or more kinds of amino acids into protein. So far only one such specificity has been found. Poly U directs small amounts of leucine as well as phenylalanine into protein. The ratio of the two amino acids incorporated is about 20 or 30 molecules of phenylalanine to one of leucine. In the absence of phenylalanine, poly U codes for leucine about half as well as it does for phenylalanine. The molecular basis of this specificity is not known. Nor is it known of the dual coding seen in living systems as well as in cellular systems.

Base sequences that do not code for any amino acid are termed "nonsense words." This term may be misleading, for such sequences, if they exist, might have meaning to the cell. For example, they might indicate the beginning or end of a portion of genetic message. An indirect estimate of the frequency of nonsense words can be obtained by comparing the efficiency of random RNA preparations with that of normal messenger RNA. We have found that many of the synthetic polymers containing three, four or five links of bases are as efficient in stimulating protein synthesis as natural polymers are. This high efficiency, together with high coding specificity, suggests that relatively few base sequences are nonsense words.

In his recent article in Scientific American, Dr. Christian Anfinsen presented arguments for believing that the coding ratio is either three or a multiple of three. Recently we have determined the relative amounts of different amino acids directed into protein by synthetic RNA preparations of known base ratios, and the evidence suggests that some code words almost surely contain three bases. Yet, at the table at the bottom of the next two pages shows, both of the 20 amino acids can be coded by words containing only two different bases. The exceptions are aspartic acid and methionine, which seem to require some combination of U, C and A. (Some uncertainty still exists about the code words for these amino acids, because even poly-UCA directs very little aspartic acid at methionine into protein.) If the entire code indeed consists of triplets, it is possible that correct coding is achieved, in some instances, when only two out of the three bases read are recognized. Such imperfect recognition might occur more often with synthetic RNA polymers containing only one or two bases than it does with natural messenger RNA, which always contains a mixture of all four. The results obtained with synthetic RNA may dem-

Two kinds of transfer RNA have been found, each capable of transporting leucine (Lys). One kind (left) recognizes the code word UUG; the other (right) recognizes UUC.

Polymer—molecules formed by combining two or more identical, or similar, smaller molecules. In this case, the polymers are used in preparing synthetic RNA.

High coding specificity—This means that one particular word codes for one particular amino acid. We may say that a particular triplet code word is "specific" for the amino acid it affects.
The code letter combinations shown here are all possible combinations of nitrogen bases if you have a singlet, doublet, or triplet code.

Mathematically, the possibilities can be shown by taking "4" (the number of different nitrogen bases in RNA) to the power which is equal to the number of bases in the coding unit. For a singlet code, $4^1 = 4$ possibilities. For a doublet code, $4^2 = 16$ possibilities. For a triplet code, $4^3 = 64$ possibilities.

As one can see from these calculations, only a triplet pattern provides enough words to code for 20 different amino acids.

**SICKLE CELL ANEMIA**—a type of anemia (blood disease) where the red blood cells look like sickles, instead of like bi-concave discs.

---

SINGLE CODE

<table>
<thead>
<tr>
<th>A</th>
<th>G</th>
<th>C</th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>AG</td>
<td>AC</td>
<td>AU</td>
</tr>
<tr>
<td>GA</td>
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<td>GC</td>
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<tr>
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<td>UG</td>
<td>UC</td>
<td>UU</td>
</tr>
</tbody>
</table>

DOUBLE CODE

<table>
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<th>G</th>
<th>C</th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAA</td>
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<td>AAC</td>
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<td>GAC</td>
<td>GUA</td>
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<tr>
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<td>CAG</td>
<td>CAC</td>
<td>CUA</td>
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<tr>
<td>UAA</td>
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<td>UUA</td>
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</table>

TRIPLET CODE

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<th>C</th>
<th>U</th>
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<td>CAG</td>
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<td>CUA</td>
</tr>
<tr>
<td>UAA</td>
<td>UAG</td>
<td>UAC</td>
<td>UUA</td>
</tr>
</tbody>
</table>

---

**COUPLE LETTER COMBINATION** increase sharply with the length of the code word. Since at least 20 code words are needed to identify the 20 common amino acids, the minimum code length is a sequence of three letters, assuming that all words are the same length.

An indirect check on the validity of code words obtained in cell-free systems can be made by studying natural proteins that differ in amino acid composition at only one point in the protein chain. For example, the hormone of an individual suffering from *sickle cell* anemia differs from normal hemoglobin in that it has valine at one point in the chain instead of glutamic acid. Another abnormal hemoglobin has lysine at the same point. One might be able to show, by examining the code-word dictionaries, that these three amino acids-glutamic acid, valine, and lysine—have similar code words. One could then infer that the two abnormal hemoglobins came into being as a result of a mutation that substituted a single base for another in the gene that controls the production of hemoglobin. As a matter of fact, the code word dictionary shows that the code words are similar enough for this to have happened. One of the code groups for glutamic acid is AGC. Substitution of a U for a C yields ACA, one of the code groups for lysine. Similar analyses have been made for other proteins in which amino acid substitutions are known, and in most cases the substitutions can be explained in the direction of a single base in code word triplets. Presumably more code words will be found in the future and the correlation between genetic base sequences and amino acid sequences can be made with greater assurance.

**The Nature of Messenger RNA**

Does each nucleotide of messenger RNA function only once or many times in directing the synthesis of protein? The question has proved difficult because most of the poly-U's in the experimental system are degraded before it is able to function as messenger. We have found, nevertheless, that only about 1.5 U's in poly-U are required to direct the incorporation of one molecule of phenylalanine into protein. And George Snell and Fritz A. Lipmann of the Rockefeller Institute have reported that only about 37 U's are required per molecule of amino acid in their studies. If the coding is done by triplets, there would be the requirement of the messenger functioning only once. Evidently each poly-U molecule directs the synthesis of more than one long-chain molecule of polyphenylalanine. Similar results have been obtained in intact cells. Cyrus Levinthal and his associates at the Massachusetts Institute of Technology.
of Technology "isolated" messenger RNA synthesis in living bacteria with the antibiotic ampicillin and found that each messenger RNA molecule present at the time messenger synthesis was turned off directed the synthesis of 10 to 20 molecules of protein.

We have observed that to factor in addition to have sequence have a profound effect on the activity of messenger RNA: the length of the RNA chain and its overall structure. Poly-U molecules that contain more than 100 U's are much more active than molecules with fewer than 50. Robert G. Martin and Bruce Ames of the National Institutes of Health have found that chains of poly-U containing 50 to 700 U's are optimal for directing protein synthesis.

There is still much to be learned about the effect of structure on RNA function. Unlike tRNA, RNA molecules are usually single-stranded. Frequently, however, one part of the RNA molecule loops back and forms hydrogen bonds with another portion of the same molecule: the exon of such internal pairing is influenced by base sequence in the molecule. Where poly-U is in isolation, it usually has little secondary structure; that is, it consists of a single chain with few, if any, loops or knots. Other types of RNA molecules display a considerable amount of secondary structure [see top illustration on page 8].

We have found that such a secondary structure interferes with the activity of messenger RNA. When solutions of poly-U and poly-A are mixed, they form double-stranded (U-A) and triple-stranded (U-A-U) helices, which are completely inactive in directing the synthesis of polyphenylalanine. Its collaboration with Maxine F. Singer of the National Institutes of Health we have shown that poly-U containing a high degree of ordered secondary structure (possibly due to hydrogen-bonding) is unable to code for amino acids. It is conceivable that natural messenger RNA contains at intervals short regions of secondary structure resembling knots in a rope. These regions might significantly affect the beginning of the coding of a protein. Alternative to polymerases suggest that the beginning and end are indicated by particular base sequences in the genetic message. In any case it seems probable that the secondary structure assumed by different types of RNA will be found to have great influence on biological function.

The Reading Mechanism

Still not completely understood is the manner in which a given amino acid finds its way to the proper site in a protein chain. Although transfer RNA was found to be required for the synthesis of polyphenylalanine, the possibility remained that the amino acid rather than the transfer RNA recognized the codon word embodied in the poly-U messenger RNA.

To distinguish between these alternative possibilities, a brilliant experiment was performed jointly by François Chapelville and Lepinex of the Rockefeller Institute, Center von Ehrenstein of Johns Hopkins University and three further workers: Reiner, Wiseman and William H. Jr. In one amino acid, cysteine, is directed into protein by poly-U. Alanine, which is identical with cysteine except that it lacks a sulfur atom, is directed into protein by poly-CG or poly-UC. Cysteine is transported by one species of transfer RNA and alanine by another. Chapelville and his associates enzymatically attached cysteine, labeled with carbon 14, to this particular type of transfer RNA. They then exposed the resulting complex to a nickel catalyst, called flayed nickel, which removed the sulfur from cysteine and converted it to alanine-without detecting it from cysteine-transfer RNA. Now they could ask: Will the labeled alanine be codified as if it were alanine or cysteine? They found it was codified by poly-UC, just as if it were cysteine [see bottom illustration on page 9]. This experiment shows that an amino acid loses its identity after combining with transfer RNA and is carried only by the code word recognized by the transfer RNA.

The secondary structure of transfer RNA itself has been elucidated further this past year by workers at King's College of the University of London. From X-ray evidence they have deduced that transfer RNA consists of a double helix very much like the secondary structure found in DNA. One difference is that the transfer RNA molecule is folded back on itself, like a hairpin that has been twisted around its long axis. The molecule seems to contain a number of unpaired bases; it is possible that these provide the means for recognizing specific code words in messenger RNA [see illustration at right on page 7].

Here is still considerable mystery about the way messenger RNA attaches to ribosomes and the part that ribosomes play in protein synthesis. It has been known for some time that eukaryotic ribosomes are composed of at least two types of subunits and that under certain conditions they form aggregates consisting of two subunits (dimers) and four subunits (tetramers). In collaboration with Samuel Baraneder, we found that the addition of poly-U to reaction mixtures initiated further ribosome aggregation. In early experiments only tetramers or still larger aggregates supported the synthesis of polyphenylalnine. Sparkes and Lipscomb have shown that poly-U makes only certain "active" ribosomes aggregate and that the remaining monomers and dimers do not support polyphenylalanine synthesis.

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# Inhibitor—kept from performing its expected function.

**HYDROGEN BONDS**—the attraction of hydrogen atoms to atoms that have a lot of electrons. In water molecules there is an attraction of the H's in one molecule to the O's in another.

**HELICES**—plural of helix.

**ALANINE**...**CYSTEINE**—Below are the structures of these two similar amino acids:

- alanine: CH3-C=CH-COOH
- cysteine: HS-CH2-C=CH-COOH

**TRANSFER RNA**—See diagrams of transfer RNA on p. 9.
```
50

AMINO ACID | RNA CODE WORDS
--- | ---
ALANINE | CCA, CCG, CCC, CAC, UCA
ARGININE | CAG, UGA
ASPARGINE | AAC, ACA
ASPARAGINE | AAA, UAA
GLUOPERIC ACID | GAA, GGC, GAC, ACA
GLUTAMINE | AAU, AAC
GLYCINE | GGU, GCA
HISTIDINE | HAA
GLUTAMIC ACID | CUA, CAC
GLUTAMIC ACID | GUA, GAC
GLYCYNE | GGC
LEUCINE | UUA, UUG, UAC
LYSINE | AAA, AAG
LYSINE | UUA, UUG
METHIONINE | UUA
PHENYLALANINE | UUA
PROLINE | CCA, CUG, CAC, UCA
SERINE | UUA, UUG
THREONINE | AAC, ACA, AUA
TRYPTOPHAN | UUA, UUG
VALINE | GUA

The Author

MARMELL W. NIRENBERG is head of the Section of Biochemical Genetics at the National Heart Institute, one of the more important institutes at the University of Florida in 1948. After receiving a B.S. in biology from the University of Florida in 1952, Nirenberg went to the department of biological chemistry at the University of Michigan, where he acquired a Ph.D. in 1957. A two-year postdoctoral fellowship from the American Cancer Society brought him to the National Institute of Arthritis and Metabolic Diseases later the same year, and he remained until he took his present post in June of last year.

Bibliography


ANNOTATORS

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INTRODUCTORY NOTE

Since the beginning of time man has wondered about the differences and similarities among living things. The first man to study heredity seriously was Gregor Mendel (1822-1884), who developed a way of predicting the appearance and traits of garden peas.

Since Mendel, geneticists and biochemists have learned a great deal about how characteristics of living organisms are passed on to their offspring. The genes, or units of heredity, that carry the information about how future organisms should be made were shown to be contained in structures called chromosomes, which are found in the nuclei of all cells.

More recently, genes were shown to be parts of specific large molecules called DNA (deoxyribonucleic acid). This molecule is composed of deoxyribose (a sugar), phosphate, and certain small molecules called nucleotide bases.

Having a molecular weight in the millions, or billions, it was very difficult to determine how the parts of this molecule were put together. At this stage scientists knew what the gene was made of, but not what it looked like or how it worked. Many studies were done using techniques like X-ray diffraction to try to determine how DNA was put together and how it did its job.

In the early 1950's, in what has been called the greatest biological discovery of the century, James Watson and Francis Crick proposed a double helix model for DNA that accounted for all the facts then known about DNA. This model provided geneticists and biochemists with the information they needed to know in order to begin serious study about just how DNA works.

Since Watson and Crick's discovery, a certain type of DNA (which is a double helix) has been shown to be circular, or joined end to end within the chromosome. The purpose of this paper is to present a theory about how circular DNA goes about dividing and forming new DNA.
Amino Acids—organic compounds composed almost entirely of carbon, hydrogen, oxygen, and nitrogen, which are the building blocks of proteins. There are only about 20 amino acids which make up all the proteins of living things. In any individual protein several hundred, or thousands, of amino acids are combined together in a specific sequence.

Proteins—a class of large complex organic molecules composed of amino acids. In living organisms, proteins function as enzymes, tissue builders, and energy sources.

DNA—abbreviation for deoxyribonucleic acid. DNA is a molecule, or molecule, in the chromosomes of the nucleus of cells which "directs" the manufacture of proteins in an organism. By controlling the production of proteins (which serve as catalysts in the making of other molecules), we can say that DNA controls, directly or indirectly, all the building and maintenance of the cells in an organism.

Every organism has its own uniquely structured DNA.

Labeled—"tagged" for the purpose of identifying something. In this study certain radioactive atoms are connected to certain parts of DNA molecules. These atoms expose film during the process of autoradiography and consequently leave a "sketch" of the molecule.

Radioactive—the adjective which describes an atom which spontaneously emits radiation (or particles) from its nucleus. Some atoms do this naturally because their nuclei are unstable. Other "normal" atoms can be made radioactive by various techniques, one of which is nuclear bombardment.

Autoradiographs—"self-portraits" of radioactive sources made by placing the radioactive material next to photographic film. The process of making these pictures is called autoradiography.

Labeled—identically copied from the original. Today biochemists and geneticists usually use the word replicate, instead of duplicate, for this activity of DNA. Replicate means "to make a copy or close reproduction."

Nucleotide Bases—important subunits of DNA. There are four nucleotide bases: adenine, guanine, cytosine, and thymine. In the early 1950's Watson and Crick showed that the structure of DNA was a spiral double chain, or "twisted ladder." The nucleotide bases make up the "rungs" of this ladder. The particular sequence of these rungs determines what kind of protein is made. (See diagram in notes on James D. Watson on this page).

Genes—thse parts of a chromosome in the cell nucleus which determine the hereditary traits. (The term gene was used long before scientists knew much about the structure of cells, hence it has had various shades of meaning as the study of "genes" progressed. "Gene" is at best a general term.)

Translated—something turned from one language into another. Here the language of nucleic acids (base sequences) is turned into (or translated into) the language of proteins (amino acid sequences). In this fashion a segment of nucleic acid gives rise to a segment of protein.

Ribonucleic Acid (RNA)—a molecule similar in structure to one-half of a DNA molecule (split down the rungs of the ladder). RNA first copies the genetic information of the DNA by a process known as transcription (conversion of a nucleotide sequence of DNA into a nucleotide sequence of RNA). The RNA then carries this copied genetic information from the DNA to the site where proteins are made. It is there where the RNA is "translated" (see above note).
THE BACTERIAL CHROMOSOME

When bacterial DNA is labeled with radioactive atoms, it takes its own picture. Autoradiographs reveal that the bacterial chromosome is a single very long DNA molecule and show how it is duplicated.

The information inherited by living things from their forebears is inscribed in their deoxyribonucleic acid (DNA). It is written there in a decipherable code, which the "letters" of which the "words" are the nucleotides, are the four subunits of DNA, the coaxial bases. It is ordered in functional units—the genes—which are translated by way of ribosomal and tRNA sequences of amino acids that determine the properties of proteins. The genes are, in the final analysis, the executors of each organism's inheritance.

The crucial event is the passage of genetic information from one generation to the next is the duplication of DNA. This cannot be a casual process. The complement of DNA in a single bacterium, for example, amounts to some six million nucleotide bases; this is the bacterium's "inheritance." Clearly life's security of tenure derives in large measure from the precision with which DNA can be duplicated, and the manner of this duplication is therefore of great consequence. This article deals with a single set of experiments on the duplication of DNA, the antecedents to them and some of the speculations they have provoked.

When James D. Watson and Francis H. C. Crick developed their two-stranded model for the structure of DNA, they saw that it contained within it the seeds of a system for self-duplication. This structure, they said, "could lead to the solution of the problem of heredity" (Watson and Crick, 1953). Now, 17 years later, it is clear that the majority of genes are organized in tandem repeats and that this kind of repetition could be the normal process of duplication, since the mechanism of DNA replication and its role in the living cell. Thus, there was the general background of the experiments I undertook.

Furthermore, the experiments required a new kind of thinking. The model of DNA as a double helix, with its two antiparallel strands, was developed to explain the structure of DNA, but it also suggested a mechanism for replication. The experiments described in this article were designed to test this mechanism and to determine how the two strands of DNA are copied during replication.

Interpretation of autoradiographs on opposite page is based on the varying density of the line of grains. Borderline autoradiographic density represents doubly labeled DNA, silver-photostimulated DNA, single-labeled DNA, and unradiaed DNA. The parent chromosomes, labeled in one strand and part of another, begin to duplicate at 4; new labeled strands have been laid down in two loops as far as 8.

For the fascinating story of this discovery see the best seller. The Double Helix: A Personal Account of the Discovery of the Structure of DNA by James D. Watson (AlbSEMUM, 1968).

POLYNUCLEOTIDE CHAINS—(See polymerization in notes on p. 7.) A polymer of nucleotides (i.e., many nucleotides joined together in a chain) is a polynucleotide. A nucleotide is composed of a nucleotide base (i.e., adenine, guanine, cytosine, and thymine in DNA, and uracil in RNA). Each strand of DNA or RNA is a "polynucleotide.""

COMPLEMENTARY BASE PAIRING—(See complement in notes above.) In DNA the "rungs" which connect the "side pieces" of the ladder are composed of pairs of nucleotide bases. According to the Watson-Crick model only two types of base pairs occur in DNA: adenine is joined to thymine (and vice-versa) and cytosine is joined to guanine (and vice-versa). In helical portions of RNA adenine pairs with uracil instead of thymine. Specific pairs of nucleotide bases are known as complementary base pairs.

TEMPLATE—"a pattern . . . used as a guide for making something accurately." (American Heritage Dictionary.)

COMPLEMENTARY CHAIN—(See notes on complement and complementary base pairing above.) For a given polynucleotide chain composed of a "side piece" and "rungs" (the nucleotide bases), only one particular chain—composed of a side piece and "rungs" —can be formed to "match up" and make a complete ladder. This is because each nucleotide base in the "side piece" can combine with one, and only one, of the other three nucleotide bases. This particular chain is called the complementary chain to the given chain. See below.

INTERPRETATION of autoradiographs on opposite page is based on the varying density of the line of grains. Borderline autoradiographic density represents doubly labeled DNA, silver-photostimulated DNA, single-labeled DNA, and unlabeled DNA. The parent chromosomes, labeled in one strand and part of another, begin to duplicate at 4; new labeled strands have been laid down in two loops as far as 8.

ENZYMES—"a protein, that without being used up or permanently changed, speeds up the rate of a specific biochemical reaction." (A compound that does this is called a catalyst.)

PRECURSOR—"one that precedes another; predecessor." (American Heritage Dictionary.) In biochemical this refers to a compound that is required to produce other compounds. Here in the article, precursors are small molecules such as deoxyribonucleic acid, phoshphate, and the nucleotide bases which are required to produce poly-nucleotides.

ARTIFACTS—man-made objects. Here this refers to the things which appear in the autoradiograph which are consequences of the experimental technique, but do not occur in nature.

DUPLEXES—"doublets." Here a duplex refers to the pair of chains of the double helix.
GENETIC MARKERS—regions in the DNA which have been identified with respect to their function.

VIRUSES—compounds made of protein and DNA (or RNA) which have the "life-like" property of being able to reproduce themselves in host cells of organisms, but cannot multiply in the absence of host tissue.

ONEROUS—troublesome, burdensome.

TOPOGRAPHICAL—the adjective for topography. Topography refers to the surface features of an object or region. This study may be considered topographical in that it primarily involves observing the surface features of DNA.

STRANDS—This refers to the "half-ladder" (split through the rung) portions, or chains, of DNA. In a complete molecule of DNA one strand was the original parent DNA and the other strand is newly synthesized DNA.

ELECTRON MICROSCOPY—a technique of magnification which uses beams of electrons to make larger images than can be produced by ordinary optical microscopy which uses light beams.

SEMICOONSERVATIVE DUPLICATION was confirmed by the Meselson-Stahl experiment, which showed that each DNA molecule is composed of two parts: one that is present in the parent molecule, the other comprising new material synthesized when the parent molecule is duplicated. If radioactive labeling begins with the first doubling, the unlabelled (black) and labeled (red) molecule chains of DNA form two-chain duplex as shown here.

DUPICATION could proceed in various ways (a-e). In these examples parental chains are shown in black lines and new chains as colored lines. The arrows show the direction of growth of the new chains, the menor parts of which are denoted by bracketlike segments.

Various factors dictated that the experiments should be conducted with E. coli. For one thing, this bacterium was known from genetic studies to have only one chromosome, that is, its DNA is contained in a single functional unit which all the genetic markers are arrayed in sequence. For another thing, the duplication of its chromosome was known to occupy virtually the entire cycle of cell division, so that one could be sure that every cell in a rapidly multiplying culture would contain replicating DNA.

Although nothing was known about the number of DNA molecules in the E. coli chromosome (or in any other complex chromosome, for that matter), the dispersal of the bacteriophage's DNA among its descendants had been shown to be semiconservative. For this and other reasons it seemed likely that the bacterial chromosome would turn out to be a single very large molecule. All the DNA previously isolated from bacteria had, to be sure, proved to be in molecules much smaller than the total chromosome, but a reason for this was suggested by studies of A. D. Hershey of the Carnegie Institution Department of Genetics at Cold Spring Harbor, N.Y. He had pointed out that the giant molecules of DNA that make up the genetic complement of certain bacterial viruses had been mixed by earlier workers simply because they are so large that they are exceedingly fragile. Perhaps the same thing was true of the bacterial chromosomes.

If so, the procedure for inspecting the replicating DNA of bacteria would have to be designed to rate for an exceptionally fragile molecule, since the bacterial chromosome contains some 20 times more DNA than the largest bacterial virus. It would have to be a case of looking but not touching. This was not an onerous restriction as it may sound. The problem was, after all, a topographical one, involving delineation of strands of parent DNA and newly synthesized DNA. There was no need for manipulation, only for visualization. Although electron microscopy is the
SPURIOUS—false, or misleading.

GENERATION-TIME—the time of the average life span of a generation. In the case of bacteria the generation-time begins with the formation of a "birth" of a daughter cell (produced by cell division) and continues until the daughter cell itself divides into two daughter cells.

MICRON—one-thousandth of a millimeter, or one-millionth of a meter.

LOCUS—place, or position.

PULS-E-LABELING EXPERIMENT—a very short time experiment. Here it refers to the labeling of DNA after exposure of only three minutes (1/10th of a generation time) to the radioactive isotope.

DNA synthesized in E. coli fed radioactive thymine for three minutes is visible in an autoradiograph, enlarged 1,200 diameters, as an array of heavy black grains (left). The events leading to the autoradiograph are shown at right. The region of the DNA chain synthesized during the "pulse-labeling" is radioactive and is shown in red (top). The radioactivity affects silver grains in the photographic emulsion (14). The developed grains appear in the autoradiograph (15), approximately delineating the new chains of DNA.

some molecules that—however big—had not been broken and see their form. In a search of E. coli synthesizes DNA during its entire division cycle, some of the extracted DNA should be caught in the act of replication. Since there was an excess of unlabelled DNA present, any tendency for DNA to produce artificial aggregations would not produce a significant increase in the size of the labeled molecules or an alteration in their form.

It is the peculiar virtue of autoradiography that one sees only what has been labeled; for this reason the technique can yield information on the history as well as the form of a labeled structure. The easiest way to determine which of the schemes of replication was correct was to look at bacterial DNA that had been allowed to duplicate for only a short time in the presence of labeled thymine. Only the most recently made DNA would be visible (corresponding to the broken-line segments in the bottom illustration on page 40), and so it should be possible to determine if the two daughter molecules were being made at the same point or in different regions of the parent molecule. A picture obtained after labeling bacteria for three minutes, or a tenth of a generation time (as left in illustration above), makes it clear that two labeled structures are being made in the same place. This place is presumably a particular region of a larger (unseen) parent molecule (see diagram at right in illustration above).

The autoradiograph also shows that at least 80 nanoseconds (80 nanoseconds of a millimeter) of DNA has been double-labeled in three minutes. Since duplication occupies the entire generation-time (which was about 20 minutes in these experiments), it follows that the process seen in the autoradiograph could traverse at least 10 to 80 nanometers, or about a millimeter, of DNA between cell divisions and the next. This is roughly the total length of the DNA in the bacterial chromosome. The autoradiograph therefore suggests that the entire chromosome may be duplicated at a single burst that can move fast enough to traverse the total length of the DNA in each generation.

Finally, the autoradiograph gives evidence on the semiconservative aspect of duplication. Two structures are being synthesized. It is possible to estimate how heavily each structure is labeled in terms of grains produced per micron of length by counting the number of exposed grains and dividing by the length. Then the density of labeling can be compared with that of virus DNA labeled similarly but uniformly, that is, in both of its polynucleotide chains. It turns out that each of the two new structures seen in the picture must be a single polynucleotide chain. If, therefore, the picture is showing the synthesis of two daughter molecules from one parent molecule, it follows that each daughter molecule must be made up of one new (labeled) chain and one old (unlabeled) chain—just as Watson and Crick predicted.

The "pulse-labeling" experiment just described yielded information on the isolated region of bacterial DNA actually engaged in duplication. To learn if the entire chromosome is a single molecule and how the process of duplication proceeds it was necessary to look at DNA that had been labeled with radioactive thymine for several generations. Moreover, it was necessary to find, in the profile of chromosomes extracted from E. coli, autoradiographs of unbroken chromosomes that were discern
tangled enough to be seen as a whole. Rather then repeat the steps that led after many molecular weight pictures of the entire bacterial chromosome in one piece, it is simpler to present two sample autoradiographs and explain how they can be interpreted and what they reveal.

The autoradiographs on page 2 and 3 show bacterial chromosomes in the process of duplication. All that is visible is labeled, or "hot", DNA, any unlabeled, or "cold", DNA is omitted. A number of DNA duplexes labeled in only one chain ("hot-cold") make a faint trace of black grains. A duplex that is doubly labeled ("hot-hot") shows as a heavier trace. The autoradiographs therefore indicate, as shown in the diagram that accompanies them, the extent to which new, labeled polynucleotide chains have been laid down along labeled or unlabeled parent chains. Such data make it possible to construct a bacterial family history showing the process of duplication over several generations [see illustration on next page].

The significant conclusions are these:

1. The chromosome of E. coli apparently contains a single molecule of DNA weighing a million meters in length and with a calculated molecular weight of about two billion. This is by far the largest molecule known to be a biological system.

2. The molecule contains two polynucleotide chains, which separate at the time of duplication.

3. The molecule is duplicated at a single focus that traverses the entire length of the molecule. At this point both new chains are being made; two chains are becoming four. This focus has come to be called the replicating fork because that is what it looks like.

4. Replicating chromosomes are not Y-shaped, as would be the case for a linear structure [see "d" in bottom illustration on page 4]. Instead the three ends of the Y are joined; the ends of the daughter molecules are joined to each other and to the far end of the parent molecule. In other words, the chromosome is circular while it is being duplicated.

It is hard to conceive of the behavior of a molecule that is about 1,000 times larger than the largest protein and that exists, moreover, coiled inside a cell several hundred times shorter than it self. Apart from this general problem of comprehension, there are two special difficulties inherent in the process of DNA duplication outlined above. Both have their origin in details of the structure of DNA that I have not yet discussed.

The first difficulty arises from the unique properties of the two polynucleotide chains [see illustration on page 4]. Each deoxyribophosphate backbone of one chain of the DNA duplex bears the sequence in the order 5'-O-P-C5'-O-P-C5'-O-P-C5'-O-P-C5'. The C5's are the three carbon atoms of the deoxyribose that contribute to the backbone. The other chain has the sequence 3'-O-P-C5'-O-P-C5'-O-P-C5'-O-P-C5'.

If both chains are having their complements laid down at a single focus moving in one particular direction, it follows that one of these new chains must grow by repeated addition to the C5's of the preceding molecule's deoxyribose ring and the other must grow by addition to a C5. One would expect that two different enzymes should be needed for these two quite different kinds of polymerization. As yet, however, the reaction that adds to chains ending in C5 has not been demonstrated in such experiments as we have performed. This fact led us to suspect a model of replication in which the two strands grow in opposite directions [see "e" and "e" in bottom illustration on page 4], where both these schemes are correct, the problem of opposite polarities remains to be explained.

The second difficulty, like the first, is related to the structure of DNA. For the sake of simplicity I have been representing the DNA duplex as a pair of chains being parallel to each other. In actuality the two chains are wound bâ¢ically around a common axis, with one complete turn for every 10 base pairs, or 24 angstrom units of length (34 base-pairs of a nucleotide). It would seem, therefore, that separation of the chains at the time of duplication, like separation of the strands of an ordinary rope, must involve rotation of the entire molecule with respect to the two daughter molecules. Moreover, this rotation must be very rapid. A fast-replicating bacterium can divide every 20 minutes.

COMPLETE CHROMOSOME is seen in this autoradiograph, enlarged about 350 diameters. Like the chromosome represented on pages 2 and 3, this one is circular, although it happens to have been labeled in a minor component shape and some segments are unlabelled. Whereas the first chromosome was more than halfway through the duplication process, this one is only about one-sixth duplicated from a to b.

OPPOSITE POLARITIES. If one numbers the steps in the sugar of one chain of DNA and goes in the same direction numbering the atoms in the other chain, one finds a reverse series as below: 

- O - C3 - C4 - C5 - O - P - C3 - C4 - C5 - O - P - 

- O - C3 - C4 - C5 - O - P - 

This reversal is referred to as opposite polarity.

BACKBONE. The backbone refers to the "sugar" of the DNA ladder.

TWO DIFFERENT ENZYMES. In most biochemical reactions enzymes are required for the reaction to occur. Enzymes are usually specific to one particular reaction. Since chain growth in addition to C3 or C5 atoms are two different reactions, one would expect that two different enzymes would be needed here.

POLYMERIZATION. The formation of a polymer. A polymer is a complex molecule of high molecular weight made up of many repeating units of simpler molecules. For example a protein is a polymer of amino acids, DNA and RNA are polymers of nucleotides. [See note on polyuridylic-acid chain on p. 33]

HELICALLY. The prefix helix. A helix is a spiral. DNA is a helical double helix or a double spiral (like a spring).

BASE PAIRS. The base pairs which form the rungs of the ladder connecting the side pieces. As mentioned earlier, these pairs are complementary, that is, each base can combine with only one other base.

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REPLICATION— making a close copy of. Here, this refers to the process where the DNA splits along the rungs of the ladder and new, complementary DNA is formed step-by-step and joined to the original DNA halves. (This process has been called duplication earlier in the paper.)

HYPOTHESIS— an assumption which accounts for a set of facts and is subject to testing and proof.

CONCENTRIC— an adjective which describes circles having the same center.

BACTERIAL DNA MOLECULE apparently replicates as in this schematic diagram. The two chains of the circular molecule are represented as concentric circles, joined at a "nucleus" (grey spot). Labeled DNA is shown in color: part of one chain of the parent molecule is labeled, as are two generations of newly synthesized DNA. Duplication starts at the nucleus and, in these diagrams, proceeds counterclockwise. The arrows mark the replicating "fork"; the point at which DNA is being synthesized in each chromosome. The drawing marked A is a schematic rendering of the chromosome in the autoradiograph on page 2 during this time it has to duplicate—and consequently to unwind—about a kilometer of DNA, or some 300,000 turns. This implies an average unwinding rate of 15,000 revolutions per minute. At first sight it may add to the difficulty to find that the chromosome is circular while all of this is going on. Obviously, a firmly closed circle—whether a molecule or a rope—cannot be unwound. This complication is worth worrying about because there is increasing evidence that the chromosome of E. coli is not exceptional in its circularity. The DNA of numerous viruses has been shown either to be circular or to become circular just before replication begins. For all we know, circularity may therefore be the rule rather than the exception.

There are several possible explanations for this apparent impasse, only one of which strikes me as plausible.

First, one should consider the possibility that there is no impasse— that in the living cell the DNA is two-stranded but not helical, perhaps being kept that way precisely by being in the form of a circle. (If a double helix cannot be unwound when it is firmly linked into a circle, neither can relational coils ever be introduced into a pair of uncircled circles.) This hypothesis requires a most improbable structure for two-stranded DNA, one that has not been observed. And it does not really avoid the unwinding problem because there would still have to be some mechanism for making nonhelical circles out of the helical ends of DNA found in certain virus particles.

Second, one could avoid the unwinding problem by postulating that at least one of the parental chains is repeated helically and resumed during replication, so that the two chains can be separated over short sections without rotation of the entire molecule. One rather sentimental objection to this hypothesis (which was proposed some time ago) is that it is hard to imagine such cavalier and hazardous treatment being voted out to such an important molecule, and one so important for its stability. A second objection is that it does not explain circularity.

The most satisfactory solution to the unwinding problem would be to find some reason why the ends of the chromosome actually must be joined together. This is the case if one postulates that there is an active mechanism for unwinding the DNA, distinct from the mechanism that copies the unwound...
OPPOSITE POLARITIES of the two parental chains of the DNA duplex result in opposite polarities and different directions of growth in the two new chains (order being held as complements of the old ones during duplication. Note that the numbered carbon atoms (1 to 5) in the deoxyribose rings (solid blocks) are in different positions in the two parental chains and therefore in the two new chains. As the replicating fork moves downward, the new chain that is complementary to the left parental chain must grow by addition to a Cp, the other new chain by addition to a Cmp, as shown by the arrows. The elliptical shapes are the four bases.

PARENTAL— the adjective for “parent.” Here this refers to the first DNA chain before it divides with each strand forming a template (pattern) for the next generation of DNA being built upon it. Hence a “parent” DNA “begats” two daughter molecules. (It is interesting to note that after replication the parent no longer exists as an individual, and each daughter is one-half parent material.)
chains. Now, any active unwinding mechanism must rotate the parent molecule with respect to the two new molecules—must hold the latter fast, in other words, just as the far end of a rope must be held if it is to be unwound. A little thought will show that this can be most surely accomplished by a machine attached directly to the parent molecule in the two daughters (see illustration below). Every turn taken by such a machine would inevitably unwind the parent molecule one turn.

Although other kinds of unwinding machine can be imagined (one could be situated, for example, at the replicating fork), a practical advantage of this particular hypothesis is that it accounts for circularity. It also makes the surprising —and testable—prediction that anyلة repairable break in the parent molecule will instantly stop DNA synthesis, no matter how far the break is from the replicating fork. If this prediction is fulfilled, and the unwinding machine acquires the responsibility that at present it lacks, we may find ourselves dealing with the first example in nature of something equivalent to a word.

GROUND— a place on the replicating molecule that is common to both the older parent DNA and the newer daughter DNA: the connecting point where the unwinding starts and ends.

DAUGHTERS— refers to the two new strands of DNA, or the new generation DNA being formed.

TORQUE— rotation or twist.

POSSIBLE MECHANISM for unwinding the DNA double helix is a replicating machine to which the end of the parent molecule and also the end of the two daughter molecules are joined. The means impacted by this machine is considered to be transmitted along the parent molecule, producing unwinding at the replicating fork. If this is correct, chromosome breaks should halt replication.
The Author

JOHN CAIRNS is director of the Cold Spring Harbor Laboratory of Quantitative Biology. He was born in England and obtained a medical degree at the University of Oxford. For several years he did research in Australia on the multiplication of influenza virus and vaccinia virus. Later he worked on the visualization of DNA molecules by autoradiography, a project he describes in part in the present article. Cairns writes that since assuming his present position he has found himself "mainly occupied with fiscal problems," although he has done some work "on the effect of breaking the bacterial chromosome on the replication of the chromosome."

Bibliography


ANNOTATORS

JOHN KNAPP II, a former Peace Corps volunteer and high school chemistry and general science teacher, graduated from Wheaton College in 1963 and earned an M.A.T. in general science from Michigan State University in 1966. He is presently completing his Ph.D. in science education at Western Michigan University in Kalamazoo, Michigan.

JOCHANAN STENESH obtained his Ph.D. degree in biochemistry in 1959 from the University of California at Berkeley. He is professor of chemistry at Western Michigan University where he is actively engaged in research on the molecular biology of proteins and nucleic acids.
Preparation of the Tests

In order to measure student understanding of the articles just described, the biochemist and the investigator prepared a twelve item, four-response, multiple-choice test for each article. Due to the brevity of the articles, that ranged from six to thirteen pages in length, the biochemist and the investigator did not attempt to force the construction of items into pre-determined categories. However, items requiring application, synthesis, and analysis were emphasized whereas items requiring recognition or recall were minimized. The items were designed to measure student understanding of the contents of the articles alone, and not that of the appended annotations. The tests were timed, and it was found that twenty minutes were sufficient for administration. Copies of the four tests developed for this study appear on the following pages.
A QUIZ ON

THE AUTOMATIC SYNTHESIS OF PROTEINS

DIRECTIONS:

Do not turn over this page until told to do so.

This is a 20-minute timed quiz designed to measure your understanding of the article, "The Automatic Synthesis of Proteins" by R. B. Merrifield. You are not expected to answer every item correctly, but work as carefully and rapidly as possible to do your best. Your score is the number of answers you mark correctly.

Please note this is an "open-book" test. If you find it necessary, you may refer to the article during the test, but since this is a timed test do not spend too much time on any single item.

Do not make any marks on your test. Write your answers with the pencil provided on the answer sheet provided. Make your marks heavy and black. If you make a mistake or wish to change an answer, be sure to erase your first choice completely.
DIRECTIONS: Mark on the IBM Answer Sheet the number that corresponds with the best answer to each of the items below. Answer the items according to the ideas discussed in the article.

1. Scientists have sought to develop automatic synthesis of proteins because this procedure
   (1) is faster than conventional methods.
   (2) is the only way to produce insulin in the laboratory.
   (3) eliminates the need for using purified amino acids.
   (4) almost exactly duplicates protein synthesis in the living cell.

2. The function of the polystyrene beads is to
   (1) become part of the protein.
   (2) add enough weight to the molecule so it will remain in place during synthesis.
   (3) transport the amino acids to the site where the protein is being synthesized.
   (4) serve as an anchor for the first amino acid in the molecule.

3. "Classical" laboratory synthesis of peptides requires all the following except
   (1) amino acids
   (2) "protecting groups"
   (3) polystyrene beads
   (4) "deprotecting groups"

4. Amino acid X is about to be joined to peptide chain Y of a synthetic protein. In order to do this, it is necessary to
   (1) "deprotect" all the reactive groups of X
   (2) "block" all but one group on Y
   (3) "deprotect" all reactive groups on X and Y
   (4) "block" all reactive groups on X and Y

5. In peptide synthesis a great excess of a particular amino acid is used when that amino acid is coupled to the peptide chain because
   (1) many molecules of the amino acid break down during the synthesis.
   (2) this forces the coupling reaction to about 100% completion.
   (3) many amino acid molecules are lost through the filter.
   (4) many amino acid molecules react with side groups on the peptide chain.

6. Usually in an organic reaction, a 90% yield is considered good, however in solid-phase synthesis a 90% yield at each step is not worthwhile because
   (1) the desired protein would not be synthesized at all.
   (2) a shortage of amino acids will lead to gaps in the peptide chain.
   (3) the polystyrene beads are inhibited by "waste" products.
   (4) it would result in a final mixture of many different proteins.
7. An important characteristic of the bond between the peptide chain and the polystyrene bead is that it
   (1) is exactly like the bond formed in the "classical" synthesis of proteins.
   (2) can be broken with each filtering and washing.
   (3) can not be broken by any of the reagents added until the protein is completed.
   (4) protects the reactive side groups of the first amino acid coupled to the polystyrene.

8. The first protein that biochemists sought to synthesize was insulin because the insulin molecule
   (1) is a small protein molecule.
   (2) contains only a few different kinds of amino acids.
   (3) does not have any interchain or intrachain disulfide bridges.
   (4) does not have a precise 3-dimensional structure.

9. Laboratory biosynthesis of proteins
   (1) closely imitates the steps of cellular biosynthesis.
   (2) uses a completely different set of reactions than cellular biosynthesis.
   (3) relies heavily upon the techniques of "protection" whereas cellular biosynthesis does not.
   (4) employs whatever techniques biochemists find that get the job done.

10. All the following are steps in solid-phase synthesis except
    (1) purification of the completed protein
    (2) isolation of intermediate peptides
    (3) washing away unwanted by-products
    (4) protection of reactive groups.

11. The laboratory synthesis described in this paper is called "solid-phase" because
    (1) liquids are not used in the reactions.
    (2) the insulin molecule exists in the solid state.
    (3) an insoluble solid holds the peptide in place.
    (4) crystallized protein is the end product.

12. In the living cell the "solid support" for protein synthesis is
    (1) the ATP molecule
    (2) the ribosome
    (3) cytochrome c
    (4) the first amino acid in the chain

---THE END---
DIRECTIONS:

Do not turn over this page until told to do so.

This is a 20-minute timed quiz designed to measure your understanding of the article, “Paleobiochemistry” by Philip H. Abelson. You are not expected to answer every item correctly, but work as carefully and rapidly as possible to do your best. Your score is the number of answers you mark correctly.

Please note this is an “open-book” test. If you find it necessary, you may refer to the article during the test, but since this is a timed test do not spend too much time on any single item.

Do not make any marks on your test. Write your answers with the pencil provided on the answer sheet provided. Make your marks heavy and black. If you make a mistake or wish to change an answer, be sure to erase your first choice completely.
1. Perhaps the most surprising idea in this paper is that
   (1) the carbon 14 method can tell the age of amino acids.
   (2) ancient organisms once contained amino acids.
   (3) amino acids can be separated by means of paper chromatography.
   (4) amino acids can last for a very long time without breaking down.

2. The source of the amino acids that have been recovered from fossil bones and shells is the
   (1) proteins which the bones and shells contained when the animals were alive.
   (2) layers of rock in which the bones and shells were deposited.
   (3) calcium carbonate fraction of the bones and shells.
   (4) proteins of muscle tissue which dissolved into the shells and bones when the organisms died.

3. The purpose of using paper chromatography in this experiment was to
   (1) color the amino acids so they could be seen.
   (2) dissolve the amino acids out of the fossils.
   (3) keep the amino acids from breaking down.
   (4) separate and identify the amino acids of the fossil.

4. Carbon 14 was used in this study to
   (1) identify the unstable amino acids.
   (2) date the clam shells.
   (3) inventory the number of amino acids still present.
   (4) color the amino acids so they would show up on paper chromatograms.

5. The paper chromatograms in the article suggest that
   (1) amino acids of fossil clams were quite different from those of modern clams.
   (2) amino acids of both ancient and modern clams are similar.
   (3) amino acids of both ancient and modern clams are purple and yellow.
   (4) proteins of fossil clams primarily contained amino acids that are rare today.

6. The author briefly mentions analyses done on oil and coal to show that
   (1) oil and coal have amino acids similar to modern clams.
   (2) oil and coal contain amino acids which no longer exist in modern time.
   (3) organic molecules, other than amino acids, have remained intact for long periods.
   (4) data on coal and oil deposits are not reliable.
7. Some common amino acids were not detected in the chromatograms of fossils because they probably
   (1) evolved only recently in geologic time.
   (2) were not originally present in the fossil clams.
   (3) have been changed into other amino acids which were detected in the chromatograms.
   (4) are more unstable than the detected amino acids.

8. Fossil S is widespread both geographically and time-wise throughout the geologic record. Which of the following specimens of S would probably contain the most fossil amino acids?
   (1) S₁ which is 25,000,000 years old
   (2) S₂ which is 35,000,000 years old
   (3) S₃ which is 100,000,000 years old
   (4) S₄ which lived during the Precambrian Era

9. Which of the following specimens of the same age would probably yield the most fossil amino acids?
   (1) S₅, found in highly folded rock deep within the earth
   (2) S₆, found in horizontal beds of black shale
   (3) S₇, found in shells whose “hard parts” were composed entirely of calcite.
   (4) S₈, found in shale in a region disturbed by past volcanic activity.

10. The author of this article considers analysis of Precambrian organic molecules to be important because this geologic era
    (1) is rich in the total number of organisms preserved.
    (2) is rich in the variety of organisms preserved.
    (3) may have fossils which contain clues to the origin of life.
    (4) has fossil bones rich in amino acids.

11. Fossil clam R, estimated to be 100,000,000 years old, has been analyzed in the laboratory and found to contain no amino acids. This probably means that
    (1) most of the original amino acids are still “locked-up” in proteins.
    (2) fossil R did not contain amino acids when it was living.
    (3) the original amino acids have been destroyed.
    (4) the missing amino acids have been incorporated into other organisms.

12. The main purpose of this article is to
    (1) survey the field of paleobiochemistry.
    (2) summarize other people’s work on fossil amino acids.
    (3) report the author's research on fossil amino acids.
    (4) explain several techniques for separating fossil amino acids.

---THE END---
Do not turn over this page until told to do so.

This is a 20-minute timed quiz designed to measure your understanding of the article, "The Genetic Code: II" by Marshall W. Nirenberg. You are not expected to answer every item correctly, but work as carefully and rapidly as possible to do your best. Your score is the number of answers you mark correctly.

Please note this is an "open-book" test. If you find it necessary, you may refer to the article during the test, but since this is a timed test do not spend too much time on any single item.

Do not make any marks on your test. Write your answers with the pencil provided on the answer sheet provided. Make your marks heavy and black. If you make a mistake or wish to change an answer, be sure to erase your first choice completely.
DIRECTIONS: Mark on the IBM Answer Sheet the number that corresponds with the best answer to each of the items below. Answer items according to the ideas discussed in the article.

1. Throughout history the term "gene" has had several different shades of meaning. In this article a gene is
   (1) an entire DNA molecule.
   (2) a triplet sequence of nucleotide bases.
   (3) that segment of DNA which contains enough information to produce a protein.
   (4) the visible expression of a particular hereditary trait.

2. Of the following, the most accurate description of the sequence of genetic activity from the gene to its final product is
   (1) DNA → protein → messenger RNA
   (2) DNA → messenger RNA → protein
   (3) Protein → messenger RNA → DNA
   (4) messenger RNA → protein → DNA

3. How does knowledge about the DNA of the colon bacillus add to the understanding about other DNA, such as human DNA?
   (1) It adds little since bacterial DNA bears little resemblance to human DNA.
   (2) It adds much since the code is essentially the same for all the species that have been studied.
   (3) It adds little since the great variety of code words makes generalizations between kinds of animals impossible.
   (4) Little can be inferred about human DNA since humans have more chromosomes than the colon bacillus.

4. If there were only 13 amino acids required to make all proteins, probably scientists would have first suspected
   (1) a singlet code
   (2) a doublet code
   (3) a triplet code
   (4) a quadruplet code

5. The discovery that synthetic RNA containing only uracil made a synthetic protein containing only one amino acid was important because it
   (1) confirmed the theory of the triplet code.
   (2) suggested a way to synthesize the DNA chain which served as a template, in this case poly-adenylic acid.
   (3) suggested that a particular amino acid was directed by a particular sequence of bases.
   (4) showed that a code word for phenylalanine was a sequence of U's.

6. This paper has not presented evidence for the
   (1) sequence of bases within a triplet.
   (2) base composition of triplets.
   (3) triplet composition which codes for the amino acid phenylalanine.
   (4) triplet nature of the genetic code.
7. The experiments described by the author did not involve
(1) the use of whole living organisms.
(2) preparation of "cell-free" extracts.
(3) enzyme preparations from bacterial cells.
(4) the addition of synthetic ribonucleic acid.

8. According to the author of the article, the genetic code
(1) shows little evidence for "degeneracy."
(2) has relatively few "nonsense triplets."
(3) has few applications beyond the colon bacillus at present.
(4) can not be studied in "cell-free" systems.

9. If "words" composed of two A's and one G (referred to in the article as AGA) code for arginine and glutamine, then
(1) the sequence A-A-G could not be a legitimate code word for arginine.
(2) the sequence A-G-A could not possibly code for a third amino acid.
(3) if the sequence A-G-A were demonstrated to code for arginine, the code word for glutamine must be some other sequence such as A-A-G.
(4) this would conflict with the author's triplet theory.

10. A segment of DNA with the base sequence G-G-A-T could serve as a template for which of the following segments of RNA?
(1) G-G-U-A
(2) C-C-T-A
(3) C-C-U-A
(4) G-G-A-T

11. Triplets containing the bases X, Y, Z and W, Y, Z have been shown to code for the amino acid M. This "degeneracy"
(1) demonstrates that something other than a triplet is coding this amino acid.
(2) implies a lack of "specificity" in protein construction.
(3) indicates that two words could direct M to a protein building site.
(4) indicates that more than two words could be found to code for M.

12. The main purpose of this article is to
(1) give a first-hand report of the author's research on the triplet code.
(2) make a case for the theory that adenine, guanine, cytosine, and thymine are "letters" in the "words" of the code.
(3) summarize information gathered by several people who have studied the genetic code.
(4) outline the function of messenger RNA.

--THE END--
A QUIZ ON
THE BACTERIAL CHROMOSOME

DIRECTIONS:

Do not turn over this page until told to do so.

This is a 20-minute timed quiz designed to measure your understanding of the article, "The Bacterial Chromosome" by John Cairns. You are not expected to answer every item correctly, but work as carefully and rapidly as possible to do your best. Your score is the number of answers you mark correctly.

Please note this is an "open-book" test. If you find it necessary, you may refer to the article during the test, but since this is a timed test do not spend too much time on any single item.

Do not make any marks on your test. Write your answers with the pencil provided on the answer sheet provided. Make your marks heavy and black. If you make a mistake or wish to change an answer, be sure to erase your first choice completely.
DIRECTIONS: Mark on the IBM Answer Sheet the number that corresponds with the best answer to each of the items below. Answer the items according to the ideas discussed in the article.

(NOTE: In this test, as well as in the article, the terms "duplication" and "replication" are used. Both describe the same process. While "duplication" is the older term— and used more in the article— "replication" is the term used more today. In this test "duplication" and "replication" mean the same thing.)

1. The author of the article chose autoradiography to "picture" the DNA because this technique
   (1) can make larger pictures than electron microscopy.
   (2) shows all the parts of the DNA molecule.
   (3) minimized the breaking up of DNA molecules.
   (4) uses radioactive atoms which speed up DNA replication.

2. A mechanism which explains the unwinding of the double helix of the DNA molecule
   (1) was demonstrated by this study.
   (2) was suggested by this study.
   (3) was already known before the study began.
   (4) is considered an unsolvable mystery since DNA has such a complex structure.

3. Tritium was used in this experiment to
   (1) cause the DNA to replicate.
   (2) make autoradiograph pictures.
   (3) help keep the DNA molecule intact.
   (4) make DNA replication slow enough for a picture to be taken.

4. What piece of information, if proved to be true, would damage the author's concept of an "unwinding machine?"
   (1) The DNA of E. coli is not one, but two circular molecules.
   (2) If the parent DNA breaks and DNA synthesis still continues.
   (3) A molecule of DNA has only 200,000 instead of 300,000 "turns."
   (4) Replication along the circle of DNA occurs at irregular speeds.

5. DNA replication involves
   (1) breakdown of the chains and distribution of the fragments among the offspring.
   (2) transmission of intact double helical DNA from one generation to another.
   (3) "semiconservative" duplication whereby each chain serves as a template for new DNA.
   (4) only one of the chains.

6. Autoradiographs of DNA are lines of dots rather than solid lines because
   (1) the DNA was broken up during the experiment.
   (2) only thymine exposed the film.
   (3) parts of DNA were too small to be detected.
   (4) high magnification frequently results in a "grainy" picture that leaves out detail.
7. The preparation of DNA for this study used a procedure that
   (1) is standard for obtaining bacterial DNA.
   (2) is specifically designed to avoid DNA breakage.
   (3) did not break up the cell wall.
   (4) omitted the use of lysozyme or detergent.

8. The following are facts or concepts that appeared in the article:
   A. the "unraveling" of a "circular rope."
   B. DNA is many times longer than the cell which contains it.
   C. The generation-time of E. coli is only about 30 minutes.
   D. a "swivel" point travelling along a circular molecule
   E. The chains of DNA have opposite polarities

   According to the author of the article, which two of these are *special* problems during the
   act of replication?
   (1) B and C
   (2) A and B
   (3) B and D
   (4) A and E

9. The DNA of E. coli in this experiment differs in form or function from the DNA of ordinary
   E. coli in what respect?
   (1) The replication time has been shortened.
   (2) A base has been modified.
   (3) Replication occurs in a closed circle.
   (4) The polarities of the chains have been reversed.

10. What structure in the replicating DNA represents the "unwinding machine?"
    (1) a tritium atom
    (2) the parent molecule of DNA
    (3) the first base pair of the daughter DNA
    (4) The author does not suggest a specific molecule(s).

11. If human DNA were demonstrated to be linear, and not circular, during replication, this
    would probably
    (1) invalidate the author's findings since all DNA must either be circular or linear.
    (2) cast serious doubts upon the "swivel" mechanism in the DNA of E. coli.
    (3) alter our present theory of the "double helix."
    (4) not change the author's hypothesis for "unwinding" in the DNA of E. coli.

12. The author's main purpose in writing this article is to
    (1) show that DNA can be replicated in a cell-free system.
    (2) challenge the "Double Helix Theory."
    (3) describe DNA replication and postulate a mechanism for this process.
    (4) measure the opposite polarities of the two parental chains of the DNA duplex.

---THE END---

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Preparation of the Student Questionnaire

In order to collect additional information necessary to answer the questions posed in this study, and in future studies, a student questionnaire was prepared by the investigator. The questionnaire was designed to collect the following information:

1. The grades students received in high-school biology and tenth-grade English.

2. The interest that students report having in biology and chemistry, when asked to compare their interests with those of other students in their class.

3. The students' evaluation of their own abilities as compared with those of other students in their class.

4. The students' evaluations of the teaching ability of their biology and chemistry teachers.

5. The time that students report having spent reading the articles from *Scientific American* used in this study.

6. The extent to which the students report having read the annotations.

7. The number of articles in *Scientific American* that students read prior to this study.

8. The desire that students have for reading articles in *Scientific American* in the future.

9. The type of article, annotated or nonannotated, that the students prefer to read.

A copy of the Student Questionnaire appears on the following page.
STUDENT QUESTIONNAIRE

Year Number ________________________________

1. My average grade for the year in biology was: (Circle the letter grade. Circle X if you have not taken biology.)
   A A- B+ B B- C+ C C- D+ D D- F X

2. My average grade for the year in English was: (Circle the letter grade. Circle X if you did not take English.)
   A A- B+ B B- C+ C C- D+ D D- F X

3. Circle the numbers of the responses which best express your interest in biology and chemistry,
   biology chemistry
   1. very interested 1. very interested
   2. above average interest 2. above average interest
   3. average interest 3. average interest
   4. below average interest 4. below average interest
   5. not interested 5. not interested

4. If you had the opportunity to select another article from Scientific American on a topic of interest to you, would you enjoy reading it (if you didn’t have to be tested on it)? Circle your response. YES NO

5. If you had to read another article from Scientific American which style of paper would you prefer? (Circle one.)
   1. the article by itself (without any notes in the margin)
   2. the article with accompanying notes in the margin

6. How would you rate your teacher as to his overall ability to teach chemistry to your class? (Circle one.)
   1 very good 2 above average 3 average 4 below average 5 poor

7. How would you rank yourself in ability compared to your chemistry students in this class? (Circle one.)
   I believe I am in the
   1. top quarter of my chemistry class
   2. second quarter of my class
   3. third quarter of my class
   4. bottom quarter of my class

8. How would you rate your biology teacher as to his overall ability to teach biology to your class? (Circle one.)
   1 very good 2 above average 3 average 4 below average 5 poor

9. About how much time did you spend reading the article that did not have any notes in the margin? (Circle one.)
   1. none, I didn’t read the article,
   2. about 15 minutes
   3. about 30 minutes
   4. about 1 hour
   5. about 2 hours
   6. more than 2 hours

10. About how much time did you spend reading the article that did have extra notes in the margin? (Circle one.)
    1. none, I didn’t read the article,
    2. about 15 minutes
    3. about 30 minutes
    4. about 1 hour
    5. about 2 hours
    6. more than 2 hours

11. In the article that had the extra notes in the margin, about what percent of the notes did you read? (Circle one.)
    1. all, or almost all, the notes
    2. about 75% of the notes
    3. about 50% of the notes
    4. about 25% of the notes
    5. none, or very few of the notes

12. Have you read any articles in Scientific American before this assignment? (Circle one.)
    1. No, I had never read an article in Scientific American before this assignment,
    2. Yes, I have read one or two previous articles in Scientific American
    3. Yes, I have read more than two previous articles in Scientific American

13. What suggestions do you have for improving the extra notes that were in the margins of one of your articles?
    (You may write on the back of this sheet if you need space.)

   ---------------------------------------------------------------------

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Preparation of the Student-Ability Classification Sheet

In order to place students into ability groups for making various comparisons, a Student-Ability Classification Sheet was prepared for the teachers whose classes participated in this study. On this sheet the teachers were asked to classify their students, placing them in quarters--top quarter, second quarter, third quarter, bottom quarter. Teachers were free to choose their own criteria for their decisions, but were encouraged to have the same numbers of students in each quarter. For example, if a teacher had sixteen students, he was instructed to place four students in each quarter. If he had eighteen students, he was instructed to place four students in each of two quarters and five students in each of the other two quarters.

In addition, each teacher was asked to identify, by means of an asterisk, those students in the top quarter who were in the top ten percent of their class. Accordingly, if a teacher had fourteen students, he was instructed to select one student to be classified in the top ten percent, and if he had fifteen or sixteen students, he was instructed to select two students to be in the top ten percent.

A copy of the Student-Ability Classification Sheet that was constructed for this study is included on the following page.
Dear Chemistry Teacher:

Thank you very much for letting your chemistry classes participate in this study.

It is my understanding, from our previous discussions, that your students consider this project as "part of their chemistry course" and will cooperate with me in this study as they do with you in their regular class assignments. For this study to succeed the students must read and interact with the articles to the best of their ability. This does not imply that your students will master the material. The articles are difficult for the average student and some students are likely to become confused or discouraged. Some students, as previous studies have indicated, may become very interested in their articles and spend a great deal of time reading them. This is all right.

The articles are designed to be read INDEPENDENTLY by your students. For purposes of this study please discourage students sharing their articles or reading them together. PLEASE DO NOT DISCUSS ANY OF THE ARTICLES DURING CLASS, or answer questions which deal with the content of the articles and tests, if you desire, for class use or your own files.)

Further, I would appreciate any help you can give in keeping the articles from being marked on or damaged. They must be used by several classes. At your discretion, students may take the articles home to read, only, please do everything you can to help me get all the articles back when I return.

In the space below (or on an attached sheet) please rank your students as to your judgment of their ability. (This need not come from their "grades"). Classify your students into quarters. Of those in the "top quarter" please indicate with an asterisk those you believe make up the "top 10%".

<table>
<thead>
<tr>
<th>TEACHER __________________________</th>
<th>CLASS ____________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Quarter</td>
<td>2nd. Quarter</td>
</tr>
<tr>
<td></td>
<td>3rd. Quarter</td>
</tr>
<tr>
<td></td>
<td>Bottom Quarter</td>
</tr>
</tbody>
</table>

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CHAPTER III

THE RESEARCH DESIGN

The purpose of this chapter is to indicate how the annotated articles, and the other materials described in Chapter II, were used to answer the questions posed in this study. The investigator will (1) report on the population and sample, (2) describe the research design and the methods used for collecting data, and (3) describe the methods used to analyze the data.

The data collected in this study consist of scores of students on the specially constructed achievement tests, the responses of students on the specially constructed Student Questionnaire, and the estimates of student ability provided by teachers on the Student Ability Classification Sheet.

The Population and the Sample

Since the main purpose of this study is to compare scores of student understanding on two different types of reading material, it was decided that it was not imperative to select students from a widely scattered population. Consequently, it was decided to limit this study to high schools not more than thirty-five miles from Kalamazoo, Michigan.

On May 6, 1971, teachers of beginning chemistry classes in fourteen schools within this region were sent letters by this investigator.
accompanied by letters of endorsement by Dr. George G. Mallinson, Dean, The Graduate College, Western Michigan University, describing the nature of the study and requesting a personal interview with teachers who wished to discuss the possibility of being included in the study. Copies of these letters appear in Appendix A. Teachers from eleven of the fourteen schools responded within two weeks, on a stamped self-addressed postcard that was included with the letters, requesting an interview with the investigator. A teacher from a twelfth school responded too late to be included in the initial series of interviews, and so did not participate in the study. The teachers, some of whom were heads of science departments acting in behalf of other teachers, who requested interviews were personally visited in their schools by the investigator during May, 1971. All the teachers who were visited agreed to participate in the study.

After initial communications with the teachers, it was decided to include, in a separate sample, four classes of "advanced chemistry" or "advanced science" for the purpose of making additional comparisons. Students in the two advanced science classes all had previously taken biology, chemistry, and physics. In a few cases some students were concurrently taking physics. The two advanced chemistry classes were second-year courses in high-school chemistry largely devoted to organic chemistry.

The final list of participants in this study included 603 high-school students enrolled in classes in beginning chemistry, advanced chemistry, or advanced science, taught by fifteen teachers in eleven high schools. Table I, that follows, indicates the categories into
which the schools, teachers, and students fall.

TABLE I
PARTICIPATING SCHOOLS, TEACHERS, AND STUDENTS

<table>
<thead>
<tr>
<th>Population</th>
<th>Numbers of Schools</th>
<th>Numbers of Classes</th>
<th>Numbers of Teachers</th>
<th>Numbers of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular Beginning Chemistry</td>
<td>10</td>
<td>26</td>
<td>12</td>
<td>556</td>
</tr>
<tr>
<td>Advanced</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>47</td>
</tr>
<tr>
<td>Total</td>
<td>11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30</td>
<td>15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>603</td>
</tr>
</tbody>
</table>

Notes:

<sup>a</sup>Three of the advanced classes were in schools where regular classes also participated.

<sup>b</sup>One of the teachers of an advanced class also taught a regular class.

Although the contents of the science articles chosen for this study were more closely allied with subject matter typically taught in high-school biology than in high-school chemistry classes, chemistry classes were chosen for the following reasons:

1. Nearly all chemistry students have had a year of high-school biology. Hence, nearly all high-school chemistry students have been introduced to biochemistry. Some high-school biology students, on the other hand, depending on the course

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materials they use, might not have been introduced to biochemistry at the time of this study, while others may have studied biochemistry earlier in the year, or be in the midst of a unit dealing with biochemical topics. Although chemistry students may have retained less of the details of their study of biochemistry from a previous biology class, they were a more uniform population.

2. For many students, biology is a terminal course in science. Chemistry students, by electing an additional year of science beyond biology, are presumably more interested in science than are typical biology students, and probably are more interested in reading and using supplementary materials such as articles from Scientific American.

The Research Design

Distribution of the Science Articles

Since the purpose of this study was to measure certain effects of reading two different types (annotated and nonannotated) of science articles, each student was given to read one annotated article, and another article on a different topic without annotation. In this manner, if one considers the annotated articles to be the "experimental" type and the same articles without annotation to be the "control" type, then every participating student contributed data to both the experimental and control facets of the study.

The four articles selected from Scientific American for this
study were labeled as follows:

- Article A: "The Automatic Synthesis of Proteins"
- Article B: "Paleobiochemistry"
- Article C: "The Genetic Code: II"
- Article D: "The Bacterial Chromosome"

These same articles containing the experimental annotation were labeled A', B', C', and D' respectively.

The four articles together with their annotated analogues, were grouped into all possible pairs of articles—such that one annotated and one nonannotated article appeared in each pair, with the exception that both types of one particular article did not appear in a pair. In this fashion it was possible to assemble twelve different possible combinations of articles. These combinations are shown in Table II.

To minimize and randomize the effect that reading one article might have had on reading and understanding another, the twelve pairs in Table II were distributed as equally as possible to the students in each class.

The Procedure

In order to allow for differences in student ability, and to make the reading of the science articles as non-threatening as possible, it was decided to permit students to read the articles they received outside the classroom, as well as in the classroom under supervision.

The procedure of the experiment was as follows:
## TABLE II

ALL POSSIBLE PAIRS OF ANNOTATED AND NONANNOTATED ARTICLES SUCH THAT A SINGLE ARTICLE DOES NOT APPEAR TWICE IN THE SAME PAIR (e.g., \( A, A' \) is not a legitimate pair.)

<table>
<thead>
<tr>
<th>Pair No.</th>
<th>Articles Included in the Pair</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( A, B' )</td>
</tr>
<tr>
<td>2</td>
<td>( A, C' )</td>
</tr>
<tr>
<td>3</td>
<td>( A, D' )</td>
</tr>
<tr>
<td>4</td>
<td>( B, A' )</td>
</tr>
<tr>
<td>5</td>
<td>( B, C' )</td>
</tr>
<tr>
<td>6</td>
<td>( B, D' )</td>
</tr>
<tr>
<td>7</td>
<td>( C, A' )</td>
</tr>
<tr>
<td>8</td>
<td>( C, B' )</td>
</tr>
<tr>
<td>9</td>
<td>( C, D' )</td>
</tr>
<tr>
<td>10</td>
<td>( D, A' )</td>
</tr>
<tr>
<td>11</td>
<td>( D, B' )</td>
</tr>
<tr>
<td>12</td>
<td>( D, C' )</td>
</tr>
</tbody>
</table>

Two periods were set aside for each class in the study for reading the articles and testing. The periods selected were two days apart—on Monday and Wednesday, or Tuesday and Thursday—during the interval October 4 - November 17, 1971.

On the first of these two-day periods the investigator spent approximately fifteen minutes introducing himself to the class, describing certain aspects of the study, and distributing the appropriate pairs of science articles to the students. The investigator recorded the names of the students and identifying numbers of the arti-
icles they received. Privately, the investigator distributed the Student Ability Classification Sheet to each teacher and explained how it was to be used. Certain questions asked by students about the study and the articles were answered by the investigator. The students were told that they were expected to read each article, and be prepared for brief quizzes on the contents of each—not including the contents of the annotation—on the second of the two-day periods. For the remainder of the period, each student was encouraged to begin reading either, or both, of the articles he received. For a complete description of the procedure followed on the first day see Appendix B.

On the second day with each class, the investigator administered the appropriate pair of twenty-minute timed tests and the questionnaire to each student. Students were permitted to refer to the articles during the tests. At the beginning of the testing period the students were divided arbitrarily into two groups of approximately equal size. One of these groups took tests on the annotated articles first, while the other took tests on the nonannotated articles first. After each student completed his two tests he filled out the Student Questionnaire. Also during the second day the investigator collected the completed Student Ability Classification Sheets from each teacher. For a more detailed description of the activities on the second day see Appendix B.

**Treatment of the Data**

The analysis of the data collected for this study is divided into two parts, (1) the analysis for ascertaining the validity of
the four tests developed for this study, and (2) the analysis that sought answers to the questions posed.

**Test Validity.** Every test item was reviewed and criticized by several high-school students and at least two science educators who taught science or science education courses in the university, in addition to the investigator and the biochemist who aided in the development of the materials for this study. Items that were revised were again submitted to the biochemist for review and approval. To ascertain that validity of the items, the investigator established the following requirements that had to be met in order to accept the validity of the tests:

First, assuming that each test was a valid measure as a whole, each item on each test should discriminate between those students scoring high and those scoring low on each test. Items that did not so discriminate were dropped from each test and responses to these items were not to be included in any student's test score. For this evaluation the investigator used the ILONG Item Analysis Procedure developed by the Testing Services of Western Michigan University.¹ In the ILONG Item Analysis every student taking the tests is assigned to Upper, Middle, or Lower groups based on two "cut-off" scores. Minimum scores to be included in the Upper Group and the Middle Group are, respectively,

\[ K_u = \bar{X} + (0.616 \times p) \quad \text{and} \quad K_m = \bar{X} - (0.616 \times p) \]

¹Testing Services Utility Programs, (mimeographed bulletin distributed by the Western Michigan University Testing Service), p. 3.
where $\bar{X}$ equals the mean of all scores and $p$ equals the standard deviation of all scores. This is equivalent to isolating the upper 27% and the lower 27% of the scores in a normal distribution.

For an item on the test to be considered valid, it must discriminate between the Upper and Lower groups. That is, when comparing the Upper Group with the Lower Group, more students in the Upper Group must select the correct response. If the Index of Discrimination equals the percent of the Upper Group selecting the correct response minus the percent of the Lower Group, then the Index of Discrimination must be positive (greater than zero and less than 100) for each item in the test. See Appendix C for a further description of the WMU ILONG Item Analysis.

Second, for the specially-developed tests to be considered valid measures of what the students read in the articles, and not what students knew previously, students who report on the Student Questionnaire that they spent fifteen minutes or more reading a particular article must score significantly higher than students who reported that they had not read that article.

Third, for the specially-constructed tests to be considered valid instruments, students with greater ability and/or experience in high-school science should have higher mean scores than students with lesser ability and/or experience in high-school science. Thus, for purposes of this study, for the tests to be considered valid, (1) mean scores of advanced students (as defined previously) who reported reading the articles must be significantly higher than the mean scores of regular chemistry students who reported reading the articles, and
mean scores of regular chemistry students in the Upper Ability Quarter who reported reading the articles must score significantly higher than students in the Bottom Ability Quarter.

Analysis of the Questions Posed in This Study. In order to compare the scores in an effort to answer the questions posed in this study, the investigator used multiple-"t" tests and reported the results in terms of probabilities of the differences in scores occurring by chance. For these analyses the investigator used programs prepared by Charles Townsend for the PDP-10 computer at Western Michigan University. For assessing the significant differences among numbers of students who responded to items in certain ways as compared with random expectancies, the investigator used the chi-square technique. In some cases the investigator will report the results from visual inspection of the data.
CHAPTER IV

THE FINDINGS

The purpose of this chapter is to present the findings of the study. The findings include (1) tallies of the scores on the specially-designed tests for measuring student understanding of the contents of the articles used in this study, (2) tallies of the responses on the Student Questionnaire, (3) data analyses that satisfy the requirements established in Chapter III for ascertaining the validity of the four tests developed for this study, and (4) data analyses that sought to answer the main questions posed in Chapter I.

The data reported in this chapter were collected from 603 students from eleven high schools that participated in this study. Data from thirty-one other students were not included for one or more of the following reasons: The student (1) was absent from school on the second day of the experiment, (2) responded in an inappropriate manner on his Student Questionnaire, or failed to respond to key items needed for data analysis, (3) responded in an inappropriate manner on his test answer sheet, or (4) was excused from class for one reason or another on either of the two days of the experiment.

Tallies of Test Scores

A tally of the test scores representing student achievement on
the four tests designed for this study appears in Table III. Since students reading both versions of the same article (annotated and nonannotated) were administered the same test, scores from the same tests appear in some cases under the heading "Nonannotated Scores" and in other cases, under "Annotated Scores." Thus, each score was tallied in one of two columns depending on the type of article the student received. As each test contained twelve items, the range of possible scores was 0 - 12.

**TABLE III**

**STUDENT SCORES ON ANNOTATED AND NONANNOTATED TESTS**

<table>
<thead>
<tr>
<th>Score</th>
<th>Nonannotated Scores</th>
<th>Annotated Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>0.7%</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>2.5%</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>5.6%</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>10.5%</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>11.9%</td>
</tr>
<tr>
<td>5</td>
<td>94</td>
<td>15.6%</td>
</tr>
<tr>
<td>6</td>
<td>84</td>
<td>13.9%</td>
</tr>
<tr>
<td>7</td>
<td>73</td>
<td>12.1%</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>7.0%</td>
</tr>
<tr>
<td>9</td>
<td>54</td>
<td>9.0%</td>
</tr>
<tr>
<td>10</td>
<td>38</td>
<td>6.3%</td>
</tr>
<tr>
<td>11</td>
<td>16</td>
<td>2.7%</td>
</tr>
<tr>
<td>12</td>
<td>14</td>
<td>2.3%</td>
</tr>
</tbody>
</table>
Tallies of Data Collected on the Student Questionnaire

Tallies of data collected from responses to the Student Questionnaire follow. The responses will be reported item by item.

Item 1. *My average grade for the year in biology was:* (Circle the letter grade. Circle X if you have not taken biology.) Given thirteen possible choices, the students responded as below:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>107</td>
<td>17.4%</td>
</tr>
<tr>
<td>A-</td>
<td>82</td>
<td>13.6%</td>
</tr>
<tr>
<td>B+</td>
<td>100</td>
<td>16.6%</td>
</tr>
<tr>
<td>B</td>
<td>133</td>
<td>21.1%</td>
</tr>
<tr>
<td>B-</td>
<td>44</td>
<td>7.3%</td>
</tr>
<tr>
<td>C+</td>
<td>35</td>
<td>5.8%</td>
</tr>
<tr>
<td>C</td>
<td>36</td>
<td>6.0%</td>
</tr>
<tr>
<td>C-</td>
<td>11</td>
<td>1.8%</td>
</tr>
<tr>
<td>D+</td>
<td>4</td>
<td>0.7%</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>0.5%</td>
</tr>
<tr>
<td>D-</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>X</td>
<td>43</td>
<td>7.1%</td>
</tr>
</tbody>
</table>

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Item 2. My average grade for the year in tenth-grade English was: (Circle the letter grade. Circle X if you did not take English.)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>133</td>
<td>22.1%</td>
</tr>
<tr>
<td>A-</td>
<td>96</td>
<td>15.9%</td>
</tr>
<tr>
<td>B</td>
<td>106</td>
<td>17.6%</td>
</tr>
<tr>
<td>B-</td>
<td>110</td>
<td>18.2%</td>
</tr>
<tr>
<td>C</td>
<td>45</td>
<td>7.5%</td>
</tr>
<tr>
<td>C-</td>
<td>23</td>
<td>3.8%</td>
</tr>
<tr>
<td>C</td>
<td>28</td>
<td>4.6%</td>
</tr>
<tr>
<td>C-</td>
<td>9</td>
<td>1.5%</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>D-</td>
<td>6</td>
<td>1.0%</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>X</td>
<td>41</td>
<td>6.8%</td>
</tr>
</tbody>
</table>

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Item 3. Circle the numbers of the responses which best express your interest in biology and chemistry. (A few students who asked by what standard, or with which group, they were to compare their interests, were told by the investigator to estimate their own interests as compared with those of other students in the class.)

<table>
<thead>
<tr>
<th>Rating</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very interested</td>
<td>178</td>
<td>29.5%</td>
</tr>
<tr>
<td>Above average interest</td>
<td>196</td>
<td>32.5%</td>
</tr>
<tr>
<td>Average interest</td>
<td>174</td>
<td>28.9%</td>
</tr>
<tr>
<td>Below average interest</td>
<td>32</td>
<td>5.3%</td>
</tr>
<tr>
<td>Not interested</td>
<td>11</td>
<td>1.8%</td>
</tr>
<tr>
<td>(No response)</td>
<td>12</td>
<td>2.0%</td>
</tr>
</tbody>
</table>
### Chemistry

<table>
<thead>
<tr>
<th>Rating</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very interested</td>
<td>92</td>
<td>15.4%</td>
</tr>
<tr>
<td>Above average interest</td>
<td>198</td>
<td>32.8%</td>
</tr>
<tr>
<td>Average interest</td>
<td>248</td>
<td>41.1%</td>
</tr>
<tr>
<td>Below average interest</td>
<td>54</td>
<td>9.0%</td>
</tr>
<tr>
<td>Not interested</td>
<td>6</td>
<td>1.0%</td>
</tr>
<tr>
<td>(No response)</td>
<td>5</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

**Item 4.** If you had an opportunity to select another article from Scientific American on a topic of interest to you, would you enjoy reading it (if you didn't have to be tested on it)?

<table>
<thead>
<tr>
<th>Response</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>422</td>
<td>70.0%</td>
</tr>
<tr>
<td>No</td>
<td>167</td>
<td>27.7%</td>
</tr>
<tr>
<td>(No response)</td>
<td>14</td>
<td>2.3%</td>
</tr>
</tbody>
</table>

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Item 5. **If you had to read another article from Scientific American which style of paper would you prefer?**

<table>
<thead>
<tr>
<th>Response</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>The article by itself (without any notes in the margin).</td>
<td>117</td>
<td>19.4%</td>
</tr>
<tr>
<td>The article with accompanying notes in the margin.</td>
<td>479</td>
<td>79.4%</td>
</tr>
<tr>
<td>(No response)</td>
<td>7</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

Item 6. **How would you rate your teacher as to his overall ability to teach chemistry to your class?**

<table>
<thead>
<tr>
<th>Response</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very good</td>
<td>218</td>
<td>36.2%</td>
</tr>
<tr>
<td>Above average</td>
<td>205</td>
<td>34.0%</td>
</tr>
<tr>
<td>Average</td>
<td>136</td>
<td>22.6%</td>
</tr>
<tr>
<td>Below average</td>
<td>28</td>
<td>4.6%</td>
</tr>
<tr>
<td>Poor</td>
<td>7</td>
<td>1.2%</td>
</tr>
<tr>
<td>(No response)</td>
<td>9</td>
<td>1.5%</td>
</tr>
</tbody>
</table>
Item 7. How would you rank yourself in ability compared to the other chemistry students in this class? I believe I am in the--

<table>
<thead>
<tr>
<th>Response</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top quarter of my chemistry class</td>
<td>169</td>
<td>28.0%</td>
</tr>
<tr>
<td>Second quarter of my class</td>
<td>259</td>
<td>43.0%</td>
</tr>
<tr>
<td>Third quarter of my class</td>
<td>120</td>
<td>19.9%</td>
</tr>
<tr>
<td>Bottom quarter of my class</td>
<td>42</td>
<td>7.0%</td>
</tr>
<tr>
<td>(No response)</td>
<td>13</td>
<td>2.2%</td>
</tr>
</tbody>
</table>

Item 8. How would you rate your biology teacher as to his overall ability to teach biology to your class?

<table>
<thead>
<tr>
<th>Response</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very good</td>
<td>223</td>
<td>37.0%</td>
</tr>
<tr>
<td>Above average</td>
<td>199</td>
<td>33.0%</td>
</tr>
<tr>
<td>Average</td>
<td>106</td>
<td>17.6%</td>
</tr>
<tr>
<td>Below average</td>
<td>25</td>
<td>4.2%</td>
</tr>
<tr>
<td>Poor</td>
<td>10</td>
<td>1.7%</td>
</tr>
<tr>
<td>(No response)</td>
<td>40</td>
<td>6.6%</td>
</tr>
</tbody>
</table>
**Item 9.** About how much time did you spend reading the article that did not have any notes in the margin?

<table>
<thead>
<tr>
<th>Response</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>None. I didn't read the article</td>
<td>47</td>
<td>7.8%</td>
</tr>
<tr>
<td>About 15 minutes</td>
<td>159</td>
<td>26.4%</td>
</tr>
<tr>
<td>About 30 minutes</td>
<td>209</td>
<td>34.7%</td>
</tr>
<tr>
<td>About 1 hour</td>
<td>149</td>
<td>24.7%</td>
</tr>
<tr>
<td>About 2 hours</td>
<td>27</td>
<td>4.5%</td>
</tr>
<tr>
<td>More than 2 hours</td>
<td>3</td>
<td>0.5%</td>
</tr>
<tr>
<td>(No response)</td>
<td>9</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

**Item 10.** About how much time did you spend reading the article that did have extra notes in the margin?

<table>
<thead>
<tr>
<th>Response</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>None. I didn't read the articles</td>
<td>51</td>
<td>8.5%</td>
</tr>
<tr>
<td>About 15 minutes</td>
<td>131</td>
<td>21.7%</td>
</tr>
<tr>
<td>About 30 minutes</td>
<td>201</td>
<td>33.3%</td>
</tr>
<tr>
<td>About 1 hour</td>
<td>171</td>
<td>28.4%</td>
</tr>
<tr>
<td>About 2 hours</td>
<td>38</td>
<td>6.3%</td>
</tr>
<tr>
<td>More than 2 hours</td>
<td>6</td>
<td>1.0%</td>
</tr>
<tr>
<td>(No response)</td>
<td>5</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

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Item 11. In the article that had the extra notes in the margin about what per cent of the notes did you read?

<table>
<thead>
<tr>
<th>Response</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>All, or about all, the notes</td>
<td>240</td>
<td>39.8%</td>
</tr>
<tr>
<td>About 75% of the notes</td>
<td>108</td>
<td>17.9%</td>
</tr>
<tr>
<td>About 50% of the notes</td>
<td>104</td>
<td>17.3%</td>
</tr>
<tr>
<td>About 25% of the notes</td>
<td>64</td>
<td>10.6%</td>
</tr>
<tr>
<td>None, or very few of the notes</td>
<td>75</td>
<td>12.4%</td>
</tr>
<tr>
<td>(No response)</td>
<td>12</td>
<td>2.0%</td>
</tr>
</tbody>
</table>

Item 12. Have you read articles in Scientific American before this assignment?

<table>
<thead>
<tr>
<th>Response</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>No, I have never read an article in Scientific American before this experiment.</td>
<td>387</td>
<td>64.2%</td>
</tr>
<tr>
<td>Yes, I have read one or two previous articles in Scientific American.</td>
<td>132</td>
<td>21.9%</td>
</tr>
<tr>
<td>Yes, I have read more than two previous articles in Scientific American</td>
<td>80</td>
<td>13.3%</td>
</tr>
<tr>
<td>(No response)</td>
<td>4</td>
<td>0.7%</td>
</tr>
</tbody>
</table>
Item 13. What suggestions do you have for improving the extra notes that were in the margins of one of your articles? A rough classification of some of the responses, together with several examples of student responses to this question, appear in Appendix D.

In addition to the items listed in the Student Questionnaire, the investigator tallied the number and percent of respondents according to sex.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>325</td>
<td>53.9%</td>
</tr>
<tr>
<td>Female</td>
<td>278</td>
<td>46.1%</td>
</tr>
</tbody>
</table>

The number of students that received each article are listed below:

<table>
<thead>
<tr>
<th>Nonannotated Articles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper Code</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
</tr>
</tbody>
</table>
### Annotated Articles

<table>
<thead>
<tr>
<th>Paper Code</th>
<th>Number of Students</th>
<th>Percent of Students</th>
</tr>
</thead>
<tbody>
<tr>
<td>A'</td>
<td>151</td>
<td>25.0%</td>
</tr>
<tr>
<td>B'</td>
<td>151</td>
<td>25.0%</td>
</tr>
<tr>
<td>C'</td>
<td>154</td>
<td>25.5%</td>
</tr>
<tr>
<td>D'</td>
<td>147</td>
<td>24.4%</td>
</tr>
</tbody>
</table>

### Test Validity

Using the Western Michigan University ILong Item Analysis, an index of discrimination was calculated for each item on each test. As stated in Chapter III, for a test item to be considered usable for this study, it should discriminate between those students receiving high test scores and those receiving low on the test of which it is a part (i.e., the index of discrimination of each item must be greater than zero and less than 100).

For every item in every test the index of discrimination was found to be positive, and consequently all items were used. The indices of discrimination for the four tests developed for this study appear in Table IV. As indicated in Chapter III, a single test was used to measure achievement on an annotated article and its nonannotated analogue (e.g., the same test was used to measure achievement on articles A and A').

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TABLE IV
INDICES OF DISCRIMINATION FOR TEST ITEMS

<table>
<thead>
<tr>
<th>Items</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>A'</td>
<td>B</td>
<td>B'</td>
</tr>
<tr>
<td>1</td>
<td>59</td>
<td>48</td>
<td>52</td>
<td>47</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>63</td>
<td>54</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>43</td>
<td>42</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>36</td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>61</td>
<td>58</td>
<td>59</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>56</td>
<td>49</td>
<td>54</td>
</tr>
<tr>
<td>7</td>
<td>73</td>
<td>66</td>
<td>49</td>
<td>53</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>58</td>
<td>49</td>
<td>51</td>
</tr>
<tr>
<td>9</td>
<td>31</td>
<td>22</td>
<td>65</td>
<td>59</td>
</tr>
<tr>
<td>10</td>
<td>56</td>
<td>54</td>
<td>61</td>
<td>49</td>
</tr>
<tr>
<td>11</td>
<td>69</td>
<td>88</td>
<td>47</td>
<td>65</td>
</tr>
<tr>
<td>12</td>
<td>46</td>
<td>55</td>
<td>41</td>
<td>47</td>
</tr>
</tbody>
</table>

Mean 52 53 50 46 44 45 48 47

The second treatment designed to ascertain validity of the tests was a comparison of the achievement scores of students who reported having spent at least fifteen minutes reading the articles with those who reported they had not read the articles. For the tests to be considered a valid measure of what the students learned from the
articles, and not what they knew previously, the "readers" should score significantly higher (.05) than the "nonreaders." For additional comparisons, the investigator classified the readers and nonreaders in two ways. The first dealt with the article that the student received, the second, the teacher's estimate of the student's ability. These comparisons appear in Tables V, VI, VII, and VIII. With these and all other comparisons that follow, unless indicated otherwise, "t" values were calculated from the differences between means, and the probability of these differences occurring by chance.

It was found that the readers scored significantly higher (.000) than the nonreaders on both types of articles. When the readers and nonreaders were further classified according to the eight articles they received, the readers scored significantly higher (.05) than the nonreaders on seven articles. For the two highest probabilities (.113 for Article C' and .051 for Article A'), if one redefines a "reader" as one who spent at least thirty minutes reading an article, then the probabilities drop to .039 and .000 respectively.

When the readers and nonreaders were further classified in quarters on the basis of their teachers' estimates of their abilities, the readers scored significantly higher (.05) than the nonreaders in the Top, 3rd., and Bottom quarters for the Nonannotated Articles, and the readers scored significantly higher (.05) than the nonreaders in the Top and Bottom quarters for the Annotated Articles. Some of the wide variabilities in the probabilities of these comparisons may be attributed to the small numbers of nonreaders included when the students are divided into small groups.
<table>
<thead>
<tr>
<th>Article</th>
<th>Reading Classification</th>
<th>Number of Students</th>
<th>Mean</th>
<th>S.D.</th>
<th>Difference Between Means</th>
<th>&quot;t&quot; Value</th>
<th>df</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>nonreaders</td>
<td>15</td>
<td>4.00</td>
<td>2.34</td>
<td>-1.29</td>
<td>-1.95</td>
<td>154</td>
<td>.026</td>
</tr>
<tr>
<td></td>
<td>readers</td>
<td>141</td>
<td>5.29</td>
<td>2.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>nonreaders</td>
<td>10</td>
<td>6.00</td>
<td>1.90</td>
<td>-2.21</td>
<td>-3.32</td>
<td>149</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>readers</td>
<td>141</td>
<td>8.21</td>
<td>2.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>nonreaders</td>
<td>12</td>
<td>4.33</td>
<td>1.65</td>
<td>-0.90</td>
<td>-1.71</td>
<td>143</td>
<td>.045</td>
</tr>
<tr>
<td></td>
<td>readers</td>
<td>133</td>
<td>5.23</td>
<td>2.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>nonreaders</td>
<td>10</td>
<td>3.90</td>
<td>2.21</td>
<td>-1.60</td>
<td>-2.10</td>
<td>140</td>
<td>.019</td>
</tr>
<tr>
<td></td>
<td>readers</td>
<td>132</td>
<td>5.50</td>
<td>2.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>All non-annotated</td>
<td>47</td>
<td>4.49</td>
<td>2.21</td>
<td>-1.57</td>
<td>-4.61</td>
<td>592</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>annotated Articles</td>
<td>547</td>
<td>6.08</td>
<td>2.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: *The total number of students in this table is 594. Nine students were omitted since they failed to report how much time they spent reading the articles.
TABLE VI
COMPARISONS OF SCORES OBTAINED BY READERS WITH THOSE OF NON-READERS ON THE ANNOTATED ARTICLES*

<table>
<thead>
<tr>
<th>Article</th>
<th>Reading Classification</th>
<th>Number of Students</th>
<th>Mean</th>
<th>S.D.</th>
<th>Difference Between Means</th>
<th>&quot;t&quot; Value</th>
<th>df</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A'</td>
<td>nonreaders</td>
<td>8</td>
<td>3.88</td>
<td>2.26</td>
<td>-1.45</td>
<td>-1.65</td>
<td>146</td>
<td>.051</td>
</tr>
<tr>
<td></td>
<td>readers</td>
<td>140</td>
<td>5.33</td>
<td>2.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B'</td>
<td>nonreaders</td>
<td>11</td>
<td>6.81</td>
<td>1.64</td>
<td>-1.21</td>
<td>-2.17</td>
<td>148</td>
<td>.016</td>
</tr>
<tr>
<td></td>
<td>readers</td>
<td>139</td>
<td>8.02</td>
<td>2.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C'</td>
<td>nonreaders</td>
<td>14</td>
<td>4.64</td>
<td>1.87</td>
<td>-.67</td>
<td>-1.22</td>
<td>152</td>
<td>.113</td>
</tr>
<tr>
<td></td>
<td>readers</td>
<td>140</td>
<td>5.31</td>
<td>1.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D'</td>
<td>nonreaders</td>
<td>18</td>
<td>3.89</td>
<td>1.56</td>
<td>-1.17</td>
<td>-2.75</td>
<td>144</td>
<td>.003</td>
</tr>
<tr>
<td></td>
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Notes:
*The total number of students in this table is 598. Five students were omitted since they failed to report how much time they spent reading the articles.

aIf readers are re-defined as students who spent thirty minutes or more reading the articles, then the revised data generates the probability .000.

bIf readers are re-defined as in a, then the revised data generates the probability .039.
### Table VII

**Comparisons of Scores Obtained by Readers in Different Ability Quarters with Those of Nonreaders on the Nonannotated Articles**

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In addition to the ability of the tests to measure understandings of what the student read, the tests, to be considered valid for this study, should not be too difficult for high-school students. Hence students with greater ability and/or experience in high-school science should score significantly higher (.05) than students with lesser ability and/or experience. Accordingly, the third treatment designed to ascertain validity of the tests was to compare the scores of "advanced students" with those of "regular chemistry" students. These comparisons are found in Tables IX and X. In this series of comparisons, and all succeeding analyses, the nonreaders are not included because the purpose of this study is to measure the effects of reading science articles.

It was found that the advanced students scored significantly higher (.000) than the regular chemistry students on both types of articles. When the students were further classified according to the eight articles they received, the advanced students scored significantly higher (.05) than the regular chemistry students on all four of the nonannotated articles, and on Articles A' and D' of the annotated articles. Again, the wide variability may be partially explained by the small number of advanced students in each group.

Consistent with the previous series of comparisons, scores of regular chemistry students in the Top Quarter of ability were compared with those of the Bottom Quarter. These comparisons are found in Tables XI and XII. It was found that the students in the Top Quarter scored significantly higher (.000) than those in the Bottom. When the students were further classified according to the eight articles
they read, the Top Quarter scored significantly higher (.01) than the Bottom Quarter for each of the four nonannotated and each of the four annotated articles.
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Notes:

\(^1\)Students enrolled in the first year of high-school chemistry.

\(^2\)Students enrolled in a second year of high-school chemistry or advanced science.
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Notes:

\(^1\) Students enrolled in the first year of high-school chemistry.

\(^2\) Students enrolled in a second year of high-school chemistry or advanced science.
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The Main Findings

Data was collected and analyzed to answer the main questions posed in Chapter I. However, data provided by the advanced students are not considered in these and subsequent analyses. These questions together with the data related to these answers appear below:

Question 1. What is the relationship between scores on the specially-designed tests when comparing the mean scores of students reading the annotated articles with those of students reading the same articles without annotation?

Significant differences (.05) in student understanding were not found when the mean scores of the two groups were compared. When the students were further classified by ability, those in the third ability quarter who read the nonannotated articles scored significantly higher (.05) than those in the third ability quarter who read the annotated articles. Significant differences were not detected between the two sets of scores in any of the three other ability quarters. The probability of the difference between means of the significant comparison having occurred by chance was .039. The probabilities of the other comparisons ranged from .06 to .46. These data appear in Table XII.

Question 2. How is the amount of time reported spent reading both types of articles related to understanding the contents of the articles?

Significant differences (.05) were not detected between the scores on the two types of articles when the students were classified accord-
ing to the time they reported having spent reading the articles. The probabilities of the differences between means having occurred by chance ranged from .058 to .248. These data appear in Table XIV.

Question 3. Do students prefer reading the annotated articles to those that are not annotated?

Of the 603 students who responded to the Student Questionnaire, 479 indicated that they preferred the annotated articles, whereas 117 indicated that they preferred the articles without annotation. Excluding the nonreaders, the advanced students, and those students who reported that they read only one of the two articles they received, of the 461 students remaining, 373 indicated that they preferred the annotated articles whereas 88 preferred the nonannotated ones.

It was found, using the chi-square technique, that the students significantly (.001) preferred to read the annotated articles to those without annotation. When the students were further classified in quarters according to ability, those in each quarter significantly (.001) preferred to read the annotated articles. The preferences expressed by students appear in Table XV.

To analyze student preference still further, the numbers of students preferring the annotated articles in each quarter were compared with the students as a whole who preferred the annotated articles. The comparison was made with a binomial testing model developed by Dr. Michael Stoline of the Western Michigan University Computer Center. It was found that students in the Bottom Ability Quarter
<table>
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<th>&quot;t&quot; Value</th>
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### TABLE XIV

**COMPARISONS OF SCORES ON BOTH ANNOTATED AND NONANNOTATED ARTICLES WHEN STUDENTS ARE CLASSIFIED BY TIME REPORTED SPENT READING THE ARTICLES**

<table>
<thead>
<tr>
<th>Reported Time Spent Reading</th>
<th>Type of Articles</th>
<th>Number of Students</th>
<th>Mean</th>
<th>S.D.</th>
<th>Difference Between Means</th>
<th>&quot;t&quot; Value</th>
<th>df</th>
<th>Probability</th>
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<td>-.70</td>
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<td>4.80</td>
<td>2.08</td>
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<td>.134</td>
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<td>.056</td>
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<td>2.42</td>
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<td>About 1 hour</td>
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<td>-.68</td>
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<td>2 hours or more</td>
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<td>2.75</td>
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<td>5.80</td>
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### Table XV

**ARTICLE PREFERENCE EXPRESSED BY STUDENTS CLASSIFIED BY ABILITY QUARTER**

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<td>80.5%</td>
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<td>121</td>
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<td>97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19.8%</td>
<td>80.2%</td>
</tr>
<tr>
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<td>117</td>
<td>26</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.2%</td>
<td>78.8%</td>
</tr>
<tr>
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<td>94</td>
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<td>80.9%</td>
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</tbody>
</table>

Significantly (.02) preferred the annotated articles as compared with the group as a whole. Significant differences were not detected when each of the other quarters was compared to the whole group. This analysis appears in Table XVI.

**Question 4.** How is achievement in science (as measured by grades received in biology classes) related to understanding both types of articles?

Using the grades the students reported having received in biology on the Student Questionnaire, the investigator grouped the thirteen possible responses into four categories—"high," "middle," "low," and "didn't take biology" according to the criteria in Table XV.
TABLE XVI

COMPARISONS OF NUMBERS OF STUDENTS IN EACH QUARTER PREFERRING THE ANNOTATED ARTICLES WITH THE AVERAGE OF ALL STUDENTS PREFERRING ANNOTATED ARTICLES

<table>
<thead>
<tr>
<th>Quarter</th>
<th>( n_i )</th>
<th>( x_i )</th>
<th>Estimate of ( p_i )</th>
<th>( z_i ) score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (top)</td>
<td>113</td>
<td>91</td>
<td>.805</td>
<td>-0.196</td>
</tr>
<tr>
<td>2</td>
<td>121</td>
<td>97</td>
<td>.802</td>
<td>-0.389</td>
</tr>
<tr>
<td>3</td>
<td>117</td>
<td>91</td>
<td>.788</td>
<td>-1.63</td>
</tr>
<tr>
<td>4 (bottom)</td>
<td>110</td>
<td>94</td>
<td>.855</td>
<td>+2.34*</td>
</tr>
<tr>
<td>All Quarters</td>
<td>461</td>
<td>373</td>
<td>.809</td>
<td></td>
</tr>
</tbody>
</table>

*significant at .02 level.

NOTES: To obtain the \( z_i \) scores the following relationship was assumed:

\[
p = \frac{n_1 p_1 + n_2 p_2 + n_3 p_3 + n_4 p_4}{n}
\]

where \( n \) for \( i = 1, 2, 3, \) and 4 equals the number of students in a quarter; \( n = n_1 + n_2 + n_3 + n_4; \) \( x_i \) for \( x_1, x_2, x_3, \) and \( x_4 \) equals the number of students in each quarter who preferred the annotated articles; \( p_i \) equals the proportion of the population in each quarter which preferred the annotated articles; \( p \) was considered to be the "average population" of all students who preferred the annotated articles; \( z_i \) was considered to be equal to

\[
z_i = \frac{nx_i}{n_i} - (x_1 + x_2 + x_3 + x_4)
\]

\[
\sqrt{\frac{n}{n_i} \left( \frac{n - n_i}{n_i} \right)^{-2} \frac{x_i (n_i - x_i)}{n_i} + \sum_{i=1}^{4} \frac{x_i}{n_i} \left( \frac{n_i - x_i}{n_i} \right)}
\]

\( z_i \) was used to test the null hypothesis, \( H_0 : p_1 = p \), against the alternative hypothesis, \( H_1 : p_1 \neq p \). It can be shown that \( z_i \) for \( i = 1, 2, 3, \) and 4 has an approximate standardized unit normal distribution when \( H_0 \) is true. Hence the normal tables are used to test the above hypotheses.

This analysis was developed by Dr. Michael Stoline of the Western Michigan University Computer Center.
Using these categories, two sets of comparisons were made. First, the scores of the "high" students were compared with those of the "middle" students, and the scores of the "middle" students were compared with those of the "low" students for each of the two types of articles. The results indicated that the high students scored significantly higher (.01) than the middle students, and the middle students scored significantly higher (.05) than the low students for both types of articles. The few students who reported that they had not taken biology scored lower than the high students, but higher than middle students. The data for this analysis appear in Table XVII.

Secondly, the investigator classified the students in the grade categories according to the type of article read and tested for significant differences between the scores they obtained on the tests. The investigator failed to detect any significant differences (.05) between the mean scores on the tests for the two types of articles for any grade category. The data from this analysis appear in Table XVIII.

Question 5. How is achievement in English (as measured by
<table>
<thead>
<tr>
<th>Type of Article</th>
<th>Biology Grade</th>
<th>Number of Students</th>
<th>Mean</th>
<th>S.D.</th>
<th>Difference Between Means</th>
<th>&quot;t&quot; Value</th>
<th>df</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-annotated Articles</td>
<td>High</td>
<td>160</td>
<td>6.64</td>
<td>2.73</td>
<td>- .92</td>
<td>-3.34</td>
<td>355</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>197</td>
<td>5.72</td>
<td>2.45</td>
<td>- .57</td>
<td>-2.01</td>
<td>312</td>
<td>.023</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>117</td>
<td>5.15</td>
<td>2.40</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Didn't take Biology</td>
<td>35</td>
<td>6.40</td>
<td>3.02</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>509</td>
<td>5.92</td>
<td>2.63</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Annotated Articles</td>
<td>High</td>
<td>155</td>
<td>6.39</td>
<td>2.67</td>
<td>- .62</td>
<td>-2.22</td>
<td>348</td>
<td>.014</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>195</td>
<td>5.77</td>
<td>2.53</td>
<td>- .74</td>
<td>-2.72</td>
<td>311</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>118</td>
<td>5.02</td>
<td>2.25</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Didn't take Biology</td>
<td>38</td>
<td>5.87</td>
<td>2.38</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>506</td>
<td>5.79</td>
<td>2.55</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Biology Grade</td>
<td>Type of Article</td>
<td>Number of Students</td>
<td>Mean</td>
<td>S.D.</td>
<td>Difference Between Means</td>
<td>&quot;t&quot; Value</td>
<td>df</td>
<td>Probability</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------</td>
<td>-------------------</td>
<td>------</td>
<td>------</td>
<td>-------------------------</td>
<td>-----------</td>
<td>-----</td>
<td>-------------</td>
</tr>
<tr>
<td>High</td>
<td>Nonannotated</td>
<td>160</td>
<td>6.64</td>
<td>2.73</td>
<td>+.25</td>
<td>+.82</td>
<td>313</td>
<td>.206</td>
</tr>
<tr>
<td></td>
<td>Annotated</td>
<td>155</td>
<td>6.39</td>
<td>2.67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>Nonannotated</td>
<td>197</td>
<td>5.72</td>
<td>2.45</td>
<td>-.04</td>
<td>-.21</td>
<td>390</td>
<td>.470</td>
</tr>
<tr>
<td></td>
<td>Annotated</td>
<td>195</td>
<td>5.76</td>
<td>2.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>Nonannotated</td>
<td>117</td>
<td>5.15</td>
<td>2.40</td>
<td>+.13</td>
<td>+.42</td>
<td>233</td>
<td>.337</td>
</tr>
<tr>
<td></td>
<td>Annotated</td>
<td>118</td>
<td>5.02</td>
<td>2.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did Not Take</td>
<td>Nonannotated</td>
<td>35</td>
<td>6.40</td>
<td>3.02</td>
<td>+.53</td>
<td>+.82</td>
<td>71</td>
<td>.208</td>
</tr>
<tr>
<td>Biology</td>
<td>Annotated</td>
<td>38</td>
<td>5.87</td>
<td>2.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Nonannotated</td>
<td>509</td>
<td>5.92</td>
<td>2.63</td>
<td>+.13</td>
<td>+.80</td>
<td>1013</td>
<td>.220</td>
</tr>
<tr>
<td></td>
<td>Annotated</td>
<td>506</td>
<td>5.79</td>
<td>2.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
grades in tenth-grade English) related to understanding both types of articles?

The investigator followed the same procedure used in Question 4 to answer this question. The English grades that the students reported having received were categorized in the same manner as were the biology grades, and the same types of comparisons were made. It was found that for both types of articles the high students scored significantly higher (.01) than the middle students. However, the investigator failed to detect significant differences (.05) between the middle students and the low students. The data for this analysis appear in Table XIX.

When the students in the grade categories were classified by type of article read, the investigator failed to detect any significant differences (.05) between scores on the two types of articles. The data for these comparisons appear in Table XX.

Question 6. How is previous experience in reading articles from Scientific American related to understanding both types of articles?

Using data from Item 12 of the Student Questionnaire, the students were divided into three groups: those who (1) never previously read an article from Scientific American, (2) read previously one or articles from Scientific American, and (3) read previously more than two articles from Scientific American.

For this analysis the investigator used the same procedure as in the two previous questions. For both types of articles signifi-
cant differences (.05) were not detected between the scores of students who reported that they had never read an article from *Scientific American* and those who reported that they had read one or two articles. The data for these comparisons appear in Table XXI.

When the students in the reading experience groups were classified by the type of article read, the investigator failed to detect any significant differences (.05) between the scores on the two types of articles. The data for these comparisons appear in Table XXII.
<table>
<thead>
<tr>
<th>Type of Article</th>
<th>English Grade</th>
<th>Number of Students</th>
<th>Mean</th>
<th>S.D.</th>
<th>Difference Between Means</th>
<th>&quot;t&quot; Value</th>
<th>df</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>199</td>
<td>6.35</td>
<td>2.65</td>
<td>+ .73</td>
<td>+ 2.72</td>
<td>384</td>
<td>.003</td>
</tr>
<tr>
<td>Non-annotated</td>
<td>Middle</td>
<td>187</td>
<td>5.62</td>
<td>2.56</td>
<td>+ .25</td>
<td>+ .78</td>
<td>271</td>
<td>.218</td>
</tr>
<tr>
<td>Articles</td>
<td>Low</td>
<td>86</td>
<td>5.37</td>
<td>2.35</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Didn't take English</td>
<td>37</td>
<td>6.46</td>
<td>2.99</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>509</td>
<td>5.92</td>
<td>2.63</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>200</td>
<td>6.30</td>
<td>2.55</td>
<td>+ .91</td>
<td>+3.57</td>
<td>382</td>
<td>.002</td>
</tr>
<tr>
<td>Annotated</td>
<td>Middle</td>
<td>184</td>
<td>5.39</td>
<td>2.49</td>
<td>+ .20</td>
<td>+ .59</td>
<td>266</td>
<td>.278</td>
</tr>
<tr>
<td>Articles</td>
<td>Low</td>
<td>84</td>
<td>5.19</td>
<td>2.54</td>
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<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Didn't take English</td>
<td>38</td>
<td>6.39</td>
<td>2.25</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>506</td>
<td>5.79</td>
<td>2.55</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>English Grade</td>
<td>Type of Article</td>
<td>Number of Students</td>
<td>Mean</td>
<td>S.D.</td>
<td>Difference Between Means</td>
<td>&quot;t&quot; Value</td>
<td>df</td>
<td>Probability</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------</td>
<td>--------------------</td>
<td>------</td>
<td>------</td>
<td>--------------------------</td>
<td>-----------</td>
<td>-----</td>
<td>-------------</td>
</tr>
<tr>
<td>High</td>
<td>Nonannotated</td>
<td>199</td>
<td>6.35</td>
<td>2.65</td>
<td>+.05</td>
<td>+.16</td>
<td>397</td>
<td>.437</td>
</tr>
<tr>
<td></td>
<td>Annotated</td>
<td>200</td>
<td>6.30</td>
<td>2.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>Nonannotated</td>
<td>187</td>
<td>5.62</td>
<td>2.58</td>
<td>+.23</td>
<td>+.89</td>
<td>369</td>
<td>.187</td>
</tr>
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<td>Annotated</td>
<td>184</td>
<td>5.39</td>
<td>2.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>Nonannotated</td>
<td>86</td>
<td>5.37</td>
<td>2.35</td>
<td>+.18</td>
<td>+.48</td>
<td>168</td>
<td>.316</td>
</tr>
<tr>
<td></td>
<td>Annotated</td>
<td>84</td>
<td>5.19</td>
<td>2.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did Not Take</td>
<td>Nonannotated</td>
<td>37</td>
<td>6.46</td>
<td>2.99</td>
<td>+.07</td>
<td>+.10</td>
<td>73</td>
<td>.460</td>
</tr>
<tr>
<td>English</td>
<td>Annotated</td>
<td>38</td>
<td>6.39</td>
<td>2.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Nonannotated</td>
<td>509</td>
<td>5.92</td>
<td>2.63</td>
<td>+.13</td>
<td>+.80</td>
<td>1013</td>
<td>.212</td>
</tr>
<tr>
<td></td>
<td>Annotated</td>
<td>506</td>
<td>5.79</td>
<td>2.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**TABLE XXI**

**COMPARISONS OF SCORES WHEN STUDENTS ARE CLASSIFIED BY READING EXPERIENCE**

<table>
<thead>
<tr>
<th>Type of Article</th>
<th>Reading Experience</th>
<th>Number of Students</th>
<th>Mean</th>
<th>S.D.</th>
<th>Difference Between Means</th>
<th>&quot;t&quot; Value</th>
<th>df</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-annotated</td>
<td>&quot;Never previously&quot;</td>
<td>345</td>
<td>5.79</td>
<td>2.62</td>
<td>-.12</td>
<td>-.49</td>
<td>457</td>
<td>.312</td>
</tr>
<tr>
<td>Articles</td>
<td>&quot;1 or 2 previously&quot;</td>
<td>114</td>
<td>5.91</td>
<td>2.33</td>
<td>-.92</td>
<td>-1.73</td>
<td>158</td>
<td>.043</td>
</tr>
<tr>
<td></td>
<td>&quot;More than 2 previously&quot;</td>
<td>46</td>
<td>6.83</td>
<td>3.23</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>505</td>
<td>5.91</td>
<td>2.64</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Annotated Articles</th>
<th>Reading Experience</th>
<th>Number of Students</th>
<th>Mean</th>
<th>S.D.</th>
<th>Difference Between Means</th>
<th>&quot;t&quot; Value</th>
<th>df</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Never previously&quot;</td>
<td>346</td>
<td>5.66</td>
<td>2.47</td>
<td>---</td>
<td>-.20</td>
<td>-.71</td>
<td>457</td>
<td>.239</td>
</tr>
<tr>
<td>&quot;1 or 2 previously&quot;</td>
<td>113</td>
<td>5.86</td>
<td>2.62</td>
<td>---</td>
<td>-.86</td>
<td>-1.69</td>
<td>154</td>
<td>.047</td>
</tr>
<tr>
<td>&quot;More than 2 previously&quot;</td>
<td>43</td>
<td>6.72</td>
<td>2.89</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>502</td>
<td>5.80</td>
<td>2.56</td>
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</table>
### TABLE XXII

**COMPARISONS OF SCORES WHEN STUDENTS ARE CLASSIFIED BY READING EXPERIENCE AND TYPE OF ARTICLE**

<table>
<thead>
<tr>
<th>Reading Experience</th>
<th>Type of Article</th>
<th>Number of Students</th>
<th>Mean</th>
<th>S.D.</th>
<th>Difference Between Means</th>
<th>&quot;t&quot;</th>
<th>Value</th>
<th>df</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Never previously&quot;</td>
<td>Nonannotated</td>
<td>345</td>
<td>5.79</td>
<td>2.62</td>
<td>+ .13</td>
<td>+ .5</td>
<td>689</td>
<td>.258</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Annotated</td>
<td>346</td>
<td>5.66</td>
<td>2.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;1 or 2 previously&quot;</td>
<td>Nonannotated</td>
<td>114</td>
<td>5.91</td>
<td>2.33</td>
<td>+ .05</td>
<td>+ .16</td>
<td>225</td>
<td>.437</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Annotated</td>
<td>113</td>
<td>5.86</td>
<td>2.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;More than 2 previously&quot;</td>
<td>Nonannotated</td>
<td>46</td>
<td>6.83</td>
<td>3.23</td>
<td>+ .11</td>
<td>+ .16</td>
<td>87</td>
<td>.437</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Annotated</td>
<td>43</td>
<td>6.72</td>
<td>2.89</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>Nonannotated</td>
<td>505</td>
<td>5.91</td>
<td>2.64</td>
<td>+ .11</td>
<td>+ .70</td>
<td>1005</td>
<td>.242</td>
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<tr>
<td></td>
<td>Annotated</td>
<td>502</td>
<td>5.80</td>
<td>2.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Introduction

Many science educators believe that articles from science journals can be used effectively for achieving the knowledge and inquiry objectives commonly accepted for courses in high-school science. Among the journals suggested, Scientific American is most frequently recommended by writers of current high-school biology textbooks for supplementary readings. Yet, in a survey conducted by this investigator of 603 chemistry and advanced science students from eleven high schools in southwestern Michigan, only 13% of the students had read more than two articles in Scientific American. When students and teachers were interviewed, the most common reason cited why articles from Scientific American were not read more often was that the articles were too technical or too difficult.

Assuming Scientific American to be a useful vehicle to achieve certain objectives in high-school science, the investigator selected articles from Scientific American and through annotation, attempted to make the articles more readable, and more usable.

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The Problem

The purpose of this study was to (1) construct a set of four annotated articles on biochemistry selected from Scientific American, and (2) compare, by means of specially-designed tests, these articles with their nonannotated analogues to determine which of the two types leads to greater understanding of the contents of the latter.

This study was aimed at eliciting answers to these questions: (1) What is the relationship between scores on the specially-designed tests when comparing the mean scores of students reading the annotated articles with those of students reading the same articles without annotation? (2) How is the amount of time reported spent reading both types of articles related to understanding the contents of the articles? (3) Do students prefer reading the annotated articles to those that are not annotated? Which students prefer which articles? (4) How is achievement in science (as measured by grades received in biology classes) related to understanding both types of articles? (5) How is achievement in English (as measured by grades received in tenth-grade English) related to understanding both types of articles? and (6) How is previous experience in reading articles from Scientific American related to understanding both types of articles?

The Method

For this study the investigator selected 603 high-school students enrolled in classes of beginning chemistry, advanced chemistry,
and advanced science, taught by fifteen teachers in eleven high schools in southwestern Michigan.

On the first day of a two-day experimental period each student received two different articles to read, one annotated and one not annotated. The students were encouraged to read the articles they received in class during the first day of the two-day period, and outside class after the first day. Two days later, on the second day of the two-day period, each student was tested on specially constructed tests designed to measure understanding of the contents of the articles he read (not including the contents of the annotations). Each student also completed a questionnaire designed to elicit certain opinions and factual information. The teacher of each class completed a Student Ability Classification Sheet designed to classify students in quarters according to the teacher's conception of their abilities in science.

The Findings

Insofar as the techniques in this study are valid, the following findings may be accepted:

1. A significant difference (.05) was not detected between the mean student achievement scores on the specially-designed tests of the students who read the annotated articles and those of the students who read the same articles without annotation.

2. An analysis was made of the test scores after classifying the students according to the time they reported having spent reading the
articles. The time classifications were 0 min., 15 min., 30 min., 1 hr., 2 hr., and more than 2 hr. Although scores on the tests generally tended to increase with the amounts of time students claimed to have spent reading the articles, significant differences (.05) were not evident between the mean test scores of students reading the annotated articles and those of students reading the same articles without annotation.

3. When students were asked the type of article they would prefer reading if given another, the responses indicated a preference for the annotated type by a ratio of more than four-to-one. When the preferences of students in the Bottom Ability Quarter were compared with those of the entire sample, it was found that significantly (.02) more of the bottom ability students preferred the annotated type.

4. In another analysis the scores obtained on both types of articles were compared when the students were classified according to their grades in high-school biology. The biology grades were classified into three groups—low, middle, and high. The mean score of each of these groups was found to be significantly (.05) higher than that of the preceding group. The few students who reported that they had not taken biology scored lower than the high group, but higher than the middle group on both types of articles. However, many of the students who had not taken biology were high-ability students who had not elected the regular tenth-grade course in biology, or had taken courses in biology or life science in the ninth grade.
When students were classified according to the type of article read, significant differences (.05) were not found between the scores of the students with the same rank in achievement in the four groups (low, middle, high, and "didn't take biology").

5. In a similar analysis, the scores obtained on both types of articles were compared when the students were classified according to grades they received in tenth-grade English. The English grades were classified into three groups—low, middle, and high. The mean scores for the high groups were found to be significantly higher (.05) than those of the middle group. However, no significant difference was detected between the mean scores of the middle and low groups.

When classified according to type of article read, significant differences (.05) were not found between the scores of students with the same rank in achievement in the four groups (low, middle, high, and "didn't take tenth-grade English").

6. For both the annotated and nonannotated scores, students who reported they had read more than two articles from Scientific American previous to this study scored significantly higher (.05) than those who reported they had read one or two articles. Significant differences (.05) were not detected between the scores of students who reported they had read only one or two articles and those who reported they had never read an article in Scientific American.
Conclusions

Insofar as the findings in this study may be accepted, the following conclusions seem defensible:

Questionnaire data:

1. Most students in high-school chemistry are not, and have not been, readers of articles in Scientific American despite many explicit and implied recommendations to use this journal. The authors of nearly every current textbook in high-school biology and several science educators who have demonstrated the usefulness of science journals for achieving certain goals, claim that Scientific American contains articles useful to, and appropriate for, high-school biology students. However, only 13% of the sample surveyed had read more than two articles in Scientific American.

2. Many high-school students, after being exposed to the articles used in this study, expressed a willingness to read articles from Scientific American in the future. Seventy percent of all students surveyed responded that they would be willing to read more articles from Scientific American after being confronted with the reading and testing of this study. Considering the possibility that some students might have been threatened by this study because some may (1) not be particularly interested in biochemistry, or (2) have forgotten much of the detail of their earlier study of topics in biochemistry, or (3) have been frustrated by the time limitation of this study, the actual percent of those willing to read more articles...
might be even higher.

3. After using the materials of this study, the students preferred reading the annotated articles to the same articles without annotation by a margin of more than four-to-one.

Test validity:

The four tests designed for this study were considered valid measures of student understanding of the four articles selected from *Scientific American* and their annotated analogues for these reasons:

1. A research biochemist judged the final version of each item in each test as a valid measure of understanding the contents of the article for which the test was designed. Further, each item on each test was reviewed by at least two science educators who taught science or science education courses in the university. Items were revised where necessary.

2. Every item in every test discriminated between students scoring high and those scoring low on each test (i.e., each item had a positive Index of Discrimination).

3. Each test, when used with both types of articles to measure student achievement, discriminated between students who reported reading the articles and those who reported they hadn't read the articles. Students who reported spending at least fifteen minutes reading a particular article scored significantly higher (.05) than those who reported they had not read the article.
4. The tests were not so difficult that they failed to discriminate between students with greater ability and/or experience in science and those with lesser ability and/or experience. For both types of articles, when the scores of the advanced students were compared with those of regular chemistry students, the former scored significantly higher (.001) than the latter. Also, in a similar comparison with regular chemistry students, those in the Top Ability Quarter scored significantly higher (.001) than those in the Bottom Ability Quarter.

The main findings:

The following conclusions may be drawn from the main findings:

1. There was no evidence that the addition of annotations to the articles selected from Scientific American contributed to significantly higher (.05) scores on the tests designed to measure understanding of the contents of these articles, although the students generally preferred the annotated articles. The failure of the presence of annotation to lead the students to greater understanding of the contents of the articles may be attributed to one or more of the following:

   (1) The students may not have needed to understand the words, concepts, and techniques that were defined or explained by the annotation in order to understand the contents of the articles.

   (2) The annotation may have been too difficult to lead the students to greater understandings.
(3) The format or style of the annotation may have discouraged the students from reading and understanding it.

However, it is recognized by this investigator that the failure to detect significant difference may be attributed to one or more possible shortcomings in this study:

(1) The tests may not have adequately measured understanding of the contents of the articles.

(2) Some students may not have studied the articles as seriously as they would have if these articles were a regular part of the science curriculum, and not a special class activity.

(3) Students, with limited time for this study, may have spent too much of that time reading the annotation, and consequently read more of the contents of the nonannotated articles than the contents of the annotated articles.

In short, it appears that some feature, or features, of the annotated articles made them more appealing, in spite of their larger size and greater volume of written material, than the articles without annotation. In replies to the open-ended item on the Student Questionnaire (Item 13) which sought feedback about the articles, the annotation, and the study itself, many students responded that they thought the annotations were helpful in one way or another. But, from the fragmentary responses to this item, the precise reasons for the preference of the annotated articles cannot be ascertained. In lieu of other explanations the investigator suggests that the annotations, whether used to any great extent or not, made the annotated articles less threatening than the articles without annotations.

The student who may believe that articles from Scientific American...
are too difficult to understand, whether this be true or not, may be reassured that the necessary explanatory aids are present and ready if they become needed.

2. Since a larger percent of the lowest-ability students prefer the annotated articles to the nonannotated ones when the Bottom Ability Quarter is compared with the students as a whole, it seems that annotated articles may be more likely to be read and used by these students.

3. Previous achievement in high-school biology, as expected, is strongly related to achievement in understanding both types of articles. This seems to indicate that the ability that leads to success in classroom science is similar to that which leads to success in understanding the contents of the articles.

4. Previous achievement in tenth-grade English, which presumably is related to a student's ability in reading, is strongly related to success in understanding the articles for higher ability students, but less strongly related for lower ability students.

5. As would be expected, previous experience in reading articles from Scientific American is related to success in understanding the contents of both types of articles. However, students who have read only one or two articles from Scientific American do not have significantly higher (.05) scores than students who have never read articles from Scientific American previous to this study.
Recommendations

If one assumes that the reading of articles from Scientific American by high-school students is a worthy goal of science education, then in view of the conclusions:

a. Students are willing to read articles from Scientific American, and

b. Students generally prefer reading specially annotated articles to articles without annotation,

the investigator recommends that additional articles from various areas of science, considered to be appropriate to student learning in high school, be selected and annotated by procedures similar to those used in this study.

In view of the apparent paradoxical findings that

a. Students, by a ratio of more than four-to-one, prefer the annotated articles to the nonannotated ones, yet

b. No significant difference was detected between the achievement of students reading the annotated articles and nonannotated ones,

the investigator makes the following recommendations:

First, an additional attempt should be made to determine if the presence of annotations in science articles is related to gains in student achievement not detected by this study. Additional investigation might include (1) measurement of gains in interest in science, or gains in the knowledge, or use, of scientific method, or process, (2) measurement of attitudes toward science, scientists, or science articles, or (3) re-measurement of student understanding of the contents of the articles, perhaps measured differently from the methods

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used in this study.

Second, an attempt should be made to determine more precisely which elements need to be identified for annotating.

Third, an attempt should be made to determine the best format and style for annotation.

Finally, the investigator recommends that in additional studies of this nature the articles developed and used be more integrated into the curriculum of the classes being tested. In so doing, he feels that the articles will be less threatening, more used, and read more carefully.
Dear (teacher's name):

Prior to my entering Western Michigan University to complete a doctoral program, I was a teacher of high school chemistry. During my work with students at the high-school level, it was obvious that supplementary readings in various areas of chemistry attracted the interest of students. However, most of the supplementary readings that were available, including those in Scientific American, were frequently too difficult to understand, and some explanation was needed in order to clarify the concepts they contained. As a result, an instructor of biochemistry at Western Michigan University and I prepared a set of supplementary readings for high-school students in the area of biochemistry. These readings consist of four articles selected from Scientific American with page-by-page marginal annotations designed to explain the concepts and terminology which seemed likely to cause difficulty with the students.

Our preliminary efforts during the development of the materials have indicated that students found the articles interesting and useful. However, the population with which we dealt was too small to draw definitive conclusions.

During the Fall Semester of the academic year 1971-72 we hope to test these materials with groups of students enrolled in high-school chemistry who have also completed a year of biology. The testing of these materials will involve a maximum of two class periods, probably three days apart. During the testing period the students will read the annotated materials and report on their reactions. No preparation will be required of either the teacher who participates in this study or his students.

Obviously, the information above is relatively sketchy and, therefore, I would appreciate the opportunity of meeting with you at your convenience in your school to discuss your possible interest in participating in the evaluation. Hopefully, this meeting may be scheduled in May 1971. It is obvious that without the help of persons such as yourself, we cannot possibly make an adequate evaluation of the effectiveness of these materials.
I am enclosing a postcard on which you can indicate your interest in having a conference with me which, of course, does not assume an obligation on your part. I hope we will be hearing from you shortly. As a matter of courtesy, I am sending a copy of this letter to the principal of your high school so that he is aware of the communication that I have sent out. If I receive a positive answer, I will contact you by telephone to arrange a meeting.

I hope we will have an opportunity to talk and work together on this effort.

Sincerely,

John A. Knapp II

JAK/bjs

Enclosure

Office Phone: (616) 383-4994
Home Phone: (616) 349-6423

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May 6, 1971

Dear (teacher's name):

I am writing this letter at the request, and in behalf, of Mr. John A. Knapp II, who is currently undertaking research at Western Michigan University concerning the use of annotated supplementary reading materials for students in high school science courses. The letter from Mr. Knapp will explain the activity in greater detail.

It is common knowledge that greater use is constantly being made of supplementary reading materials in high school science and in other areas. However, research on the effectiveness of such materials is sparse.

I hope sincerely that you will be able to give strong consideration to assisting Mr. Knapp in carrying out the investigation. The results, of course, will be made known to you when the data are analyzed.

Very truly yours,

George G. Mallinson, Dean

GGM/bjs
APPENDIX B

ACTIVITIES OF THE TWO-DAY EXPERIMENTAL PERIOD
ACTIVITIES OF THE TWO-DAY EXPERIMENTAL PERIOD

The First Day: Monday (or Tuesday)

The investigator spent the first fifteen minutes introducing himself to the class and describing certain aspects of the study. The students were told that the purpose of this study was to determine how much they could learn from reading certain articles from Scientific American. Although they would not receive a grade for their efforts, they were to consider this study as a "regular part" of their class activity. All the teachers had agreed previously to consider this study "an important part" of the students' science experiences, and to encourage their students to cooperate with the aims of the experiment.

After the introduction each student received his packet of materials. The students were then given the following information verbally:

Each of you has an envelope containing two articles from the Scientific American. Please put your name on the label of your envelope. Also please record the ten-digit number on your envelope on the sheet of paper I will pass around.

Today you will spend the rest of this period reading the two articles in your envelope. You will keep these articles until Wednesday (or Thursday) when I will return.

Some of you will find the articles quite unfamiliar and difficult. Nonetheless, I expect each of you to read them and understand them to the best of your ability. You may spend as much time reading them as you like, and you may take them home if your teacher agrees. As you are only responsible for the contents of the two articles you have, please do not read them
together or discuss them in class.

When I return on Wednesday (or Thursday) I will give you a brief quiz designed to measure your understanding of the basic concepts in each of your two articles.

For the remainder of the period the students began the reading of the articles.

The Second Day: Wednesday (or Thursday)

On the second day, at the beginning of the period, each student received two tests designed to measure understanding of the contents of the two articles he had received, two standardized answer sheets designed to be scored by machine, and a copy of the Student Questionnaire. The students were then told that the tests would be "open-book" variety and that they could use their two articles if they needed them. Students who failed to bring their articles to class were provided with fresh articles. The class was then arbitrarily divided into two groups, one half on the left side of the room, the other on the right. One half was then instructed to take the tests on the annotated articles first, and the other half those on the non-annotated articles first. The students were told that when they finished the first of their two tests they were not to begin the second until the investigator so indicated, but that they could begin filling out the Student Questionnaire.

The students were informed that each test was timed for twenty minutes. At a signal from the investigator, the students began the appropriate tests. After twenty minutes, the answer sheets were
collected, and the students then began their second tests. At the end of the second twenty-minute period the investigator collected the answer sheets.

The students were allowed to keep their Student Questionnaires until the end of the class period. At the end the investigator collected the Student Questionnaires and all other materials from the students.

Before leaving the school on the second day the investigator collected the Student-Ability Classification Sheets from each participating teacher.
APPENDIX C

ITEM ANALYSIS USED IN THE STUDY (ILONG)

Reproduced with appropriate modifications from Testing Services Utility Programs, Western Michigan University, Kalamazoo, Michigan 49001
The purpose of this item analysis is to provide basic counts for test question validation. Based on a student's score, he is assigned to the Upper, Middle or Lower Group based on two "cut-off" scores.

\[
K_u = X > (0.616 \times p) \quad K_m = X - (0.616Xp)
\]

where \( X \) = Mean of all scores; \( p \) = Standard deviation of all scores.

The student's response to each question is then counted according to his answer values-1, 2, 3, 4, 5, or BL(ank). Referring to Appendix b, 115 students were in the upper group and only 82 in the lower; this is not unusual since WMU test grades tend to provide skewed "curves". The following is a frequently used procedure for evaluation of test questions.

Divide the responses into 4 categories based on upper and lower groups, done by ignoring the students within .616 standard deviations from the mean. This is equivalent to isolating the upper and lower 27 percentiles where distribution is normal. Also, whether the responses are correct or not. (answer is at top)

Group 1. Upper group, right answer (94 students)
If less than 50% of the upper group answer correctly, the question may be too difficult.

Group 2. Upper group, wrong answers (115-94 = 21)
Refer back to the test question to analyze the alternatives chosen. If many BL ank responses occur, it may indicate an ambiguous rather than a difficult question.

Group 3. Lower group, right answer (21 students)
Unless the question is intentionally easy, the percent (25) of the lower group answering correctly should be significantly less than the percent (82) of the upper group answering correctly. The difference (57) is the index of discrimination.

Group 4. Lower group, wrong answers (82-21 = 61 students)
Note the very effective "distractor" choice, answer 1 where 35% of the lower group select a particular wrong answer. Where there is a time limit on a test and not all students have time to answer every question, as the percent BL ank in the lower group nears 50%, response patterns often become meaningless since some students may quickly fill out answers to chance a few points.

Testing Services is prepared to assist faculty in evaluation of their output in respect to the actual questions used. If the available system is not acceptable, Testing Services can assist in obtaining the desired results from tests, questionnaires, and research data utilizing specialized forms and programs not described herein.
APPENDIX D

RESPONSE TO ITEM 13
ON THE STUDENT QUESTIONNAIRE
RESPONSE TO ITEM 13
ON THE STUDENT QUESTIONNAIRE

Item 13 that appeared on the Student Questionnaire was as follows:

13. What suggestions do you have for improving the extra notes that were in the margins of one of your articles? (You may write on the back of this sheet if you need space.)

In addition, all students were encouraged verbally by the investigator to record any other responses they had to the articles, the annotations, or the study.

Approximately one-half of the students responded to this item. A classification of their open-ended responses appears in Table XXIII. Students making more than one type of response are recorded more than once in the table. Nonspecific responses such as, "I liked the articles" do not appear in the table.
### TABLE XXIII

CLASSIFICATION OF RESPONSES TO
ITEM 13 ON THE STUDENT QUESTIONNAIRE

<table>
<thead>
<tr>
<th>Classification of Open-ended Response</th>
<th>Number of Students Responding</th>
</tr>
</thead>
<tbody>
<tr>
<td>The notes, as they appeared in the articles, were quite useful in understanding the contents of the articles.</td>
<td>95</td>
</tr>
<tr>
<td>Many of the notes were too difficult.</td>
<td>31</td>
</tr>
<tr>
<td>More notes should be included, or the notes which appeared should be more detailed.</td>
<td>34</td>
</tr>
<tr>
<td>Fewer notes should be included, or the notes which appeared should be less detailed.</td>
<td>21</td>
</tr>
<tr>
<td>Generally, the articles were too difficult.</td>
<td>12</td>
</tr>
<tr>
<td>The contents of the articles and/or the annotations were too boring.</td>
<td>6</td>
</tr>
<tr>
<td>&quot;I spent too much time on the notes, and not enough on the contents of the article.&quot;</td>
<td>5</td>
</tr>
<tr>
<td>&quot;The notes interfere with my way of learning.&quot;</td>
<td>5</td>
</tr>
<tr>
<td>&quot;I couldn't have understood the articles without the notes.&quot;</td>
<td>4</td>
</tr>
<tr>
<td>Other suggestions for improving the notes*</td>
<td>82</td>
</tr>
</tbody>
</table>

*This includes a wide variety of responses, many of which contradict one another. Examples of responses in this classification are: (1) The notes should be in red, or italicized. (2) The notes should appear within the body of the article, and not in the margin. (3) Equations and structural formulae should be (a) expanded, or explained more fully, (b) deleted altogether. (4) More pictures are needed.
Examples of several student responses appear below:

"... the notes added greatly to the understanding of the article, and answered questions that arose, and gave greater interest to the article."

"The information given in the notes probably would not have been sought had it not been given in the article. . . ."

"... I found it so frustrating that it was very boring. . . ."

"The notes were fine. I wish I had notes for the other article."


Ihde, Aaron J., "Learning the Scientific Method through the Historical Approach." School Science and Mathematics, LIII (November 1953), 638.


Testing Services Utility Programs', (mimeographed bulletin distributed by the Western Michigan University Testing Service).