

PREFACE

To W.C. Röntgen 100 years later



As mentioned in the May issue of the Rigaku Journal, 1995 is the 100th anniversary of the discovery of X rays and the 150th anniversary of the birth of the man who discovered them. It is therefore the time to remember the experiment and the attendant serendipitous observation by Röntgen that led to the discovery of X rays. Since 1895 the uses of X rays have been widespread, and many major areas of investigation have benefited greatly from them. Examples are provided in medical technology, in X-ray diffraction studies of crystalline and semicrystalline materials, and in studies of the structural integrity of solid materials.

The success of Wilhelm Conrad Röntgen (1845-1923) in discovering X rays hinged on his great powers of observation and deduction. Of great interest at the time was the problem of electrical conductivity in gases which are very poor conductors. The apparatus used consisted of a glass bulb (tube), fitted with platinum electrodes, that was evacuated and a large potential was applied across the two electrodes. A discharge in the form of a spark will pass through the gas if a high enough potential is used. What was found was that in these Geissler tubes (named after the glassblower) a narrow bluish beam extended from the negative electrode (the cathode) towards the positive electrode (the anode) consisting of a stream of rays, known as cathode rays. These rays caused, as shown by Julius Plücker in 1858, a green fluorescence on a small area on the glass wall of the discharge tube. In 1869 Johann Wilhelm Hittorf demonstrated that cathode rays traveled in straight lines and William Crookes showed that the location of the fluorescence on the discharge tube wall could be moved in response to a magnet. Crookes also showed, using a windmill, that the rays had momentum, implying that they were particles. Jean Baptiste Perrin found that an insulated metal cylinder acquired a negative charge when cathode rays entered it. Thus it was concluded that cathode rays are not electromagnetic in nature, but are negatively charged particles which we now know as electrons.

In 1878 Sir William Crookes designed an improved discharge tube (referred to as the Hittorf-Crookes tube). Then in 1892 Heinrich Hertz showed that cathode rays could pass through thin metal foils. This latter experiment was repeated by Philipp Lenard in 1894. He constructed a tube (the Lenard tube) with a thin aluminum window and showed that cathode rays passed through the window, but did not penetrate far in air. These last two experiments could have led to the discovery of X rays by Hertz or by Lenard since they had produced them, but these scientists failed to make detailed enough observations of the emitted radiation.

Against this background Röntgen repeated experiments already done. He knew that cathode rays emitted during discharge caused the glass walls of the tube to luminesce, but even more intense luminescence is produced by the rays when they hit a screen that is coated with certain chemicals, particularly barium platinocyanide, used to detect ultraviolet radiation. Hittorf-Crookes tubes were made of thicker glass and did not have an aluminum window as Lenard tubes did. Röntgen wondered if the cathode rays could pass through the thicker glass and produce fluorescence on a barium platinocyanide-covered screen. This was the design of the famous experiment. He covered a Hittorf-Crookes tube with a

black cardboard cover similar to one he had used with a Lenard tube and darkened the room in order to check if any radiation from the Hittorf-Crookes tube penetrated the black cardboard cover. He started up the induction coil in the darkened room so that a high tension discharge passed through the tube. No visible (fluorescent) light passed through the cardboard cover of the tube. He was going to interrupt the current in order to align the fluorescent detection screen when suddenly he noticed a weak light shimmering in the distance. This light, which was apparent only because Röntgen's eyes were dark-adapted, appeared only when the discharge was in action. Röntgen lit a match and found that the light came from a barium platinocyanide screen lying on the nearby bench more than a meter away. Since it was already known that cathode rays could only penetrate a few centimeters of air, Röntgen knew he was dealing with new type of radiation. He proved that whatever caused this effect originated from the Hittorf-Crookes vacuum tube, and he concluded that it was some form of penetrating radiation to which he gave the name X rays. This was his important discovery-noticing the luminescence on the distant screen and making the deduction that the effects of cathode rays (electrons) should be separated from those of X rays. He also demonstrated some important properties of these new rays that he had discovered. Use could be made of the varying degrees of transparency to X rays of the different compositions with materials, such as bone and flesh. When Röntgen placed his hand between the X-ray tube and the detection screen, shadows of his bones could be seen. Since X rays affected photographic film, the picture could be recorded as was a photograph of his wife's hand, complete with wedding ring, taken on December 22, 1895.

The original observation took place on November 8, 1895. Röntgen said "I discovered by chance rays that penetrated black paper." Years later, when he was asked what he thought when he discovered X rays, he is reported to have replied "I didn't think, I experimented." Indeed the next six weeks were spent investigating this physical phenomenon, and on December 28, 1895 his first paper was published entitled "Über eine neue Art von Strahlen" ("On a New Kind of Rays"), in the *Sitzungsberichte der Würzburger Physikalischen-Medicinischen Gesellschaft*. This article was immediately reprinted in a more generally available journal, and a translation appeared in *Nature* within one month of the original publication. Within a year of the first announcement of the discovery of X rays over a thousand articles on the subject had appeared in the scientific literature. The reader is urged to look at a translation of the two original articles in "Great Experiments in Physics, Firsthand Accounts from Galileo to Einstein", edited by M. H. Shamos and published by Dover Publications in New York in 1959. In 1901 Röntgen was awarded the first Nobel Prize in physics for his discovery.

Röntgen described many of the basic properties of X rays in his 1895 publications. He showed that X rays were generated whenever the cathode rays struck any solid target. Production of X rays was more efficient if the target was a heavy metal, platinum being better than aluminum as a target. This led to the subsequent design by others of more efficient X-ray tubes. Röntgen showed that X rays travel in straight lines and can cause shadows, but could not be deflected by a magnet and hence are not the same as cathode rays. Many materials such as photographic emulsions were affected by X rays. Barium platinocyanide, calcium salts, uranium glass, and rock salt each emit light (fluoresce) when exposed to X rays. He also showed that X rays can ionize gases. All substances were shown to be more or less transparent to X rays. In increasing order of transparency Röntgen listed lead glass, aluminum and wood. This variation in transparency, of course, has had numerous medical implications, already alluded to. Röntgen did not observe reflection or refraction of X rays and therefore he concluded that X rays could not be focused by lenses.

It is now 100 years since this first experiment and the world of science is vastly different because of it. Röntgen would not have believed the impact of his discovery even though the world appreciated it at the time. I will concentrate on the physical rather than the medical advances in the uses of X rays.

The physical properties of X rays that Röntgen listed in his original articles still hold. He was correct that a lens for X rays would be difficult to make and none has yet been constructed except possibly for long wavelength (10-100 Å) X rays. Grazing-incidence mirrors have been used in the past, but now microfabricated Fresnel zone plates, with a resolving power of several hundred Å, are being used. Current research in soft X-ray imaging includes efforts to obtain images in three dimensions, eliminating any loss of resolution that results from the specimen thickness. Many are working on this problem because then we could build a supermicroscope and look at atoms in molecules. However, because of the availability of X rays for use as an experimental tool, we now have a vast store of information on the arrangements of atoms in molecules, just as if it had been possible to look directly at them through a supermicroscope.

Historically, after Röntgen's work, attempts were made to determine whether X rays are waves or particles. These resulted in experiments in 1912 in which X-ray diffraction by crystalline materials, such as sodium chloride, demonstrated the wave-like properties of X rays. This result is interesting because known particles such as electrons and neutrons can also be diffracted, implying that they have some wave-like as well as particle-like character. But the important outcome was that, within a year of the discovery of X-ray diffraction, by W. Lawrence Bragg showed for simple crystal structures, that the diffraction pattern could be analyzed to give a picture of the scattering material, the electrons around atoms, and hence the three-dimensional shape of the molecule. Bragg used the earlier work of scientists like Albert Porter to show how the intensities of the diffracted beams gave information on the amplitudes of the waves to be summed in a Fourier series to give an electron-density map, an image of the electrons that scattered the X rays. The phase problem, which defined the relative phases of these summed waves, needed however to be solved. Larger molecular structures were hard to determine. For example, it was not until 1929 that the structure of hexamethylbenzene was determined by Kathleen Lonsdale, showing that the benzene molecule is flat and symmetrical. Three subsequent developments have made it possible to determine larger structures. These are the Patterson function, direct methods and the method of isomorphous replacement. The Patterson function made it possible in 1934 to determine larger crystal structures if a heavy atom was present in the crystal structure. The crystal and molecular structures of the phthalocyanines and vitamin B₁₂ were determined in this way in the 1940s and 1950s. Direct methods were developed in the 1950s and 1960s and are based on the mathematical constraints of the phases of the waves in a Fourier summation so that the electron density is positive or zero (never negative) in the electron-density maps. This method has been of great use for determining molecular structures of small- and medium-sized molecules. The method of isomorphous replacement depends on the use of the difference in diffraction intensities when two crystalline solids differ only by the identity of one atom, while the arrangement of atoms is the same in each. The intensity changes can be used to help solve the phase problem. This method, together with some derived from Patterson methods, is now successfully used to determine crystal structures of biological molecules, where the isomorphism is between the native protein or nucleic acid and its heavy-atom derivative. Finally, it is now possible to determine the absolute configuration of molecules by the use of diffraction with X rays of a wavelength that responds to an anomalously scattering atom present in the crystal.

One area of X-ray diffraction studies that is progressing at an amazing pace at this time is the study of molecules of biological interest. Every week in the major scientific journals such as *Science* and *Nature* there are reports of the three-dimensional structures of newly discovered or recently crystallized large molecules. The science of biochemistry has been revolutionized by this. When I started as a graduate student doing X-ray crystallography it was known that biological macromolecules, including viruses, could be crystallized, and that the atomic arrangement from unit cell to unit cell was similar enough for a massive number of X-ray diffraction data to be measurable. But the magnitude of the phase problem and the lack of availability of any means for doing the extensive required mathematical calculations made it

impossible at that time to calculate any electron-density map. There were many bottlenecks in crystal structure determinations, particularly the growth of crystals of adequate quality for X-ray diffraction studies, the measurement and analysis of diffraction intensities, the determination of the relative phase angles of each measured diffraction peak, the calculation of electron-density maps, and the refinement of the proposed crystal structure to obtain the best possible fit between observed and calculated data. These were time-consuming processes but they are now streamlined as a result of better instrumental and computational facilities.

Sources of X rays have greatly changed and the methods used to detect them are now very sophisticated. X rays are still produced by the acceleration of electrons at a high voltage against a metal target. Generally the X rays so produced have a wide range of wavelength, but when the target anode is a metal, X rays with wavelengths characteristic of the element are produced with a relatively high intensity, as was recognized by Charles Glover Barkla in 1911. William David Coolidge designed an improved X-ray tube in 1913 and, shortly after that a rotating anode X-ray generator in which the electron beam is directed to the outer edge of a rapidly rotating target, so that any heat generated may be dissipated more efficiently. This provides a more intense source of X rays. Another source of X rays is in the synchrotron radiation emitted when very high-energy electrons are accelerated in a magnetic field in a storage ring. Synchrotron radiation is far more intense than other sources of radiation. It is polychromatic, but required wavelengths can be selected (turned). It is therefore possible to measure diffraction data at a variety of wavelengths and, if an anomalous scatter is present, measurements of the diffraction pattern at wavelengths at which anomalous dispersion does and does not occur will provide information that can aid in the solution of the phase problem.

A development that could not have been envisioned in 1895 that has had a major effect on science in general was the manufacture of high-speed computers. The first programmable computer was the Analytical Engine designed by Charles Babbage in 1834, based on some principles used in the Jacquard loom. But it was not until the 20th century, when electrical circuits that could be switched on and off by means of vacuum tubes and magnetic circuits (and later by semiconductors) became available, that the modern computer became an item that every scientific investigator had access to. In the 1950s most laboratories had available machines that could read data from punched cards and that could add numbers together. If one needed to subtract numbers one had to fool the computer. Large computers, each filling several large rooms, were available at a few university locations and the crystallographers made as much use of them as possible. Developing computer technology, resulting from the needs of the space age, has greatly aided the crystallographer in the quest for data on molecular structures. The crystallographer, as a major user, has also aided in the development of computers. The primary problem of summing Fourier series was tackled by Beevers-Lipson strips with sine or cosine functions at defined intervals (such as $1/60$ the unit cell). These were useful but onerous to use. By 1940 the information on these strips were converted to punched cards for use with a tabulating machine. Also analog computers, such as X-RAC built by Ray Pepinsky, were used. But the most important advance was the programmable digital computer. The first all-electronic computer was ENIAC built by John W. Mauchly and J. Presper Eckert in 1946. It contained 18,000 vacuum tubes and 1,500 relays. Then followed many computers such a SWAC (National Bureau of Standards Western Automatic Computer) at UCLA and EDSAC (Electronic Delay Storage Automatic Calculator) Cambridge University in England. The first digital computer application to protein structure determination (hemoglobin) was made by John Bennett and John Kendrew on EDSAC in 1951 in response to a call for help from Hugh E. Huxley who had complained to his friend Bennett about the chore of the calculations at that time. The next revolution occurred when the vacuum tube was replaced by the transistor and integrated circuits. This led to a greater reliability and speed at a lower price. At this stage many computer programs, generally in FORTRAN, were written by the crystallographer. Now that the World-wide Web is operative one can search for and obtain programs and crystal structures results at will. But the

most important feature of present-day computing is the power of the computer that one can have in one's own laboratory to store and manipulate data, calculate electron-density maps, refine structures, and analyze results.

Originally photographic film was used for the detection of the diffraction pattern. This was used to measure diffraction photographs of proteins in 1934 and beyond and it provides excellent detection device. The extent of blackening of film could be estimated by eye versus a calibration strip or by a film-scanning photometer. Scintillation counters, Geiger counters, and proportional counters have also been used to detect X rays. Unfortunately proteins and other macromolecules contain so many atoms that the diffraction pattern to atomic resolution would have so many Bragg reflections to measure that data measurement would take years. Now methods for collecting data are streamlined by the position-sensitive area detectors which are essentially electronic film. Area detectors in use include multiwire proportional counters, television area detectors and imaging plates. These latter are storage phosphors in which a latent image of the X-ray diffraction pattern is exposed to laser light, causing the emission of light of a different wavelength which is converted by a photomultiplier into an electrical signal. Information can be stored for a considerable period of time on image plates, or the diffraction data can be read on to a computer for direct manipulation.

The method depends on the ability to grow diffraction-quality crystals. This part of the crystal-structure analysis is still fraught with uncertainty. The crystallographer has also had to learn how to purify and handle fragile biological materials, and how to find the best conditions for growing diffraction-quality crystals. Now the crystallographer studies the effects of minor variations in pH, concentration, etc. so that the best conditions for crystallization can be determined. Robotic crystallization equipment is now available and crystallization experiments have even been done on the space shuttle. The first X-ray diffraction photographs of proteins were taken in 1934 by J. D. Bernal and Dorothy Crowfoot (Hodgkin). They found out how important it was to bathe the crystal in its mother liquor. Sophisticated crystal mounting procedures are now used, but this part of the analysis is still fraught with uncertainty.

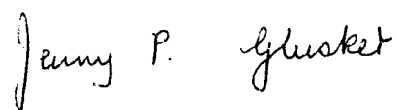
Computer graphics are now used for displaying and interpreting the electron-density map. The original program used to produce diagrams of molecular and crystal structures determined by X-ray diffraction was ORTEP (Oak Ridge Thermal Ellipsoid Plotter) written by Carroll K. Johnson in 1965. This used a computer and a pen plotter. The electron-density maps of proteins were drawn on large transparent sheets appropriately spaced to give a three-dimensional map. The fitting of a wire model of the protein, however, was very difficult until, in 1968, Frederic Richards invented a half-mirrored system affectionately known as "Fred's Folly" which allowed one to build the molecular model beside the stacks of electron density sheets while comparing both through this mirror system. This is all now done by a computer graphics program. Three-dimensional computer graphics started with Project MAC (Multiple Access Computers/machine-aided cognition) built at MIT in the 1960s. The aim was to make it easier for humans to interface to computers particularly by means of interactive graphics. The orientation of a three-dimensional object on a screen could be controlled by the user. This has been extended and programs such as 'FRODO' and "O" are available for fitting a model to an electron density map which is represented as a wire model. Programs have been written in various laboratories to represent atomic connectivity and for showing stereoviews of the structure. Many programs now produce color stereodiagrams of the macromolecule, some parts being represented diagrammatically and others showing the atomic structure. Finally the macromolecular crystallographer needs to interpret and report the structural results. The computer helps particularly by the use of diagrams and by keeping track of the millions of pieces of data measured and derived.

The ultimate aim of the X-ray diffraction study of biological macromolecules is to find the mechanistic role of the macromolecule. For this purpose Laue methods with synchrotron radiation have been used. In

Laue photography a polychromatic beam of X rays from a synchrotron source falls on a stationary crystal and the diffraction pattern is recorded on a computer. Because the exposure times are so short it is also possible to do time-resolved crystallography, that is, to measure X-ray intensities as they change in response to some change in a crystal such as the progress of a chemical reaction within the crystal. This has been successfully used to image the reactions catalyzed in crystalline glycogen phosphorylase b, and in several other proteins. Each X-ray diffraction data set provides an instantaneous snapshot of the transformation of substrate to product within the crystal, thereby establishing beyond doubt the location of the active site of the enzyme.

Now in 1995 we can view with wonder the development of X rays as radiation that interacts with electrons around atoms and reveals information on their arrangement in space. We honor Röntgen for this magnificent legacy.

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A handwritten signature in cursive script that reads "Jenny P. Glusker".

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