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The Revolution in Crystallography

Automation and computers have made x-ray structure determination a routine laboratory tool.

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The determination of the geometry of several protein molecules by x-ray diffraction and the award of the Nobel prize for such work and for the structure determination of other large molecules provide well-publicized testimony to the fact that this technique is remarkable for the study of chemical binding and molecular architecture (see *1*, *2*). Not perhaps so widely publicized is the growth that has taken place in the application of crystallography to the structure determination of molecules of small and intermediate size.

In all fields of chemistry, there has been an increasing concern with the three-dimensional conformation of molecules and the ways in which the conformation affects the equilibrium and kinetic properties of the molecules. X-ray diffraction provides the best tool for the study of the details of molecular conformation. Diffraction studies of the structures of complex inorganic and organometallic molecules have played a large part in the renaissance of inorganic chemistry, and the routine application of crystallography to organic chemistry has been an enormous aid to structure elucidation and synthesis of large molecules with interesting and useful properties.

The journal *Acta Crystallographica*, which contains many of the definitive papers on crystal structure, has (along with most other scientific journals) undergone a phenomenal increase in size over the last several years. Furthermore, many papers on crystal structure are now being published in chemical journals—for in most cases the details of the method of solving the structure are far less interesting than the chemical problems to which the technique is applied. Recent issues of *Inorganic Chemistry*, for example, have had crystal structure determination at the heart of 20 percent of the papers (Fig. 1).

Not only has the number of papers on crystal structure increased, but the size of molecule susceptible to attack by the technique has increased (Table 1). Aside from the few protein structures that have been determined (*3*), one of the largest molecules to have a complete structure determination is vitamin B_{12} (*2*), a diagram of which is shown in Fig. 2a. Structures with up to 100 atoms per molecule can usually be obtained in a routine way.

The statistics just cited are but one facet of the revolution in crystallography. Although the emphasis of this article is on the determination of crystal structures, technological and theoretical advances in crystallography have contributed significantly to many other important problems of the solid state—problems ranging from mineralogical evolution to the theory of second-order phase transformations. The crystallographer is a solid state scientist, and his interests are as broad as all of science.

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How Fast and How Much?

It is the theme of this article that the molecular structures of a large number of molecules of moderate size may now be determined quickly enough and accurately enough so that all the geometrical information necessary for the analysis of a complex chemical problem may be obtained in a reasonable period of time.

What is a "reasonable period of time" and what is the cost for carrying out a structure determination for a medium-sized molecule by crystallographic techniques? By structure determination we mean the location relative to a Cartesian axis system of all nonhydrogen atoms to a precision of 0.01 Å (bond lengths to about ½ percent) so that the conformation of the molecule is known and so that bond lengths and angles are well enough known for sensible statements to be made regarding bond order, strain, and distortions from expected geometries.

It is possible to carry out a complete crystal structure determination to obtain results of chemical significance in less than 2 weeks. This has been demonstrated in a number of laboratories but has been the goal of the "Rapid Organic Structure Analysis" project at the California Institute of Technology, in which organic chemists—in consultation with a trained crystallographer—have been learning how to carry out crystal structure determinations. The purpose of the project has been well described by Bordner (*4*), the instigator of the project:

> There is no reason why organic chemists and biochemists should not routinely use crystal structure analysis in their study of synthetic intermediates and small biological molecules . . . If x-ray analysis is to be used routinely, it must be sufficiently rapid that it will be used in the middle of a series of synthetic steps, or during the study of a metabolic pathway. If the chemist can isolate the compound, crystallize it, and determine its structure in two or three weeks, then the information from the structure analysis will be valuable in subsequent stages of his investigation.

The molecule shown in Fig. 2b is typical of those that are important to

Table 1. Number of independent atoms in molecular structures reported in *Acta Crystallographica* and *Inorganic Chemistry*. Random samples of about 30 structures per year.

Year	Journal	No. of atoms		
		Smallest structure	Average structure	Largest structure
1955	*Acta Cryst.*	2	9	21
1959	*Acta Cryst.*	3	10	21
1964	*Acta Cryst.*	3	17	37
1969	*Acta Cryst.*	4	19	52
1969	*Inorg. Chem.*	5	27	212*

* Fourteen individual atoms were refined together with 18 phenyl groups with assumed internal geometry [B. R. Davis, N. C. Payne, J. A. Ibers, *Inorg. Chem.* **8**, 2719 (1969)].

real problems in organic chemistry and whose structures were determined in an average of 2 weeks each. A structure determination carried out in such a short time may not be adequate when fine details of atomic motion and electron density are of primary interest. But it will usually be sufficient to answer a chemical problem, and it will have been done quickly enough to be of some use to the chemist.

The structures solved in the Caltech project were almost all heavy-atom derivatives, so that the structure solutions were extremely routine. This is frequently not the case; and, for large problems with no heavy atoms, a more typical elapsed time for an experienced crystallographer from growth of the crystals to first report of the results is probably something like 2 months. I know of no crystallographer who is turning out structures at the 2-week interval on a year-round basis, although there are a very few doing six structures per year. It should be noted that the "consultation with a trained crystallographer" is an important part of the Caltech project. Furthermore, although many structure solutions may be routine, problems of pseudosymmetry, twinning, disorder, and crystal stability may cause a single structure determination to become a major research project. Only a well-trained crystallographer of some years experience can on the average be expected to approach even the more pessimistic rate of six structures per year.

The cost of carrying out a crystal structure study depends on many variables. If a structure can be solved in 2 weeks, the overhead and salary costs are of course much less than the maintenance costs of a crystallographer doing six structures or less per year. In Table 2 I have presented various estimates of the cost of determining some typical crystal structures. One example is taken from the Caltech program, and two, from our experience at Brookhaven. In each case, a basic assumption is that there is a requirement for a structure solution, a refinement of the bond lengths to an estimated standard deviation of 1 picometer (0.01 Å) and preparation of the results for publication. A team of crystallographers only doing service structure analysis may well approach the Caltech ideal. However, the best structural crystallographers—those possibly capable of this level of output—are not technicians but chemists or physicists interested in particular chemical or physical problems to which they apply their crystallographic expertise. The throughput of structures for such scientists will be relatively small, but the cost estimate in column c is probably not an unfair one for each crystal structure investigation they carry out. Although Table 2 indicates that crystallographic structure determination is not inexpensive, the fact remains that it is an available tool which may provide the cheapest and most essential information for any chemical problem where precise knowledge of molecular configuration is essential. It is possible for the chemist to say, "What half-dozen structures are critical for the solution of this chemical problem?"

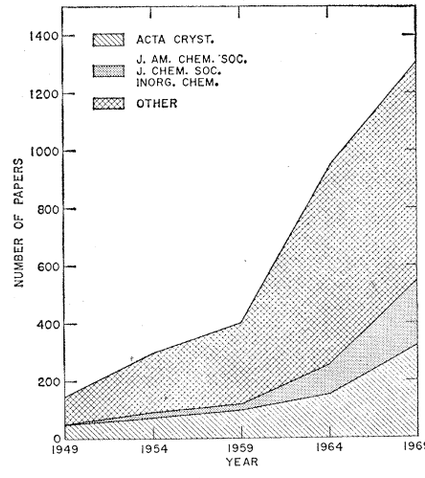

Fig. 1. Growth in number of single crystal diffraction studies as represented by a few of the best-recognized journals for reporting such work. The total number is taken as one-half the number of listings in *Chemical Abstracts* under the index entry "crystal structure."

and to then entice a willing crystallographic colleague into the laboratory to obtain the answers.

It is the purpose of this article to examine the technological advances that have made this revolution in crystallography possible. These are two: the automatic diffractometer for data collection, and the high-speed, large-memory digital computer for the structure solution and for the refinement of the parameters describing a crystal and molecular structure.

Measurement Problem

X-rays are reflected by a crystal only when the crystal is oriented such that a set of planes with Miller indices (h, k, l) makes an angle θ with both the incident and reflected beams; this angle θ is given by the Bragg condition

$$\sin\theta = (1/d)\ (\lambda/2) \qquad (1)$$

where d is the interplanar spacing and λ is the x-ray wavelength.

The intensity of each such Bragg reflection is proportional to the square of the structure factor

$$F(h,k,l) = \sum_j f_j(h,k,l)T_j(h,k,l) \exp\left[2\pi i(hx_j + ky_j + lz_j)\right] \qquad (2)$$

where the sum is over all atoms j in the unit cell of the crystal (*5*). Each atom is characterized by its atomic scattering amplitude f_j, its mean posi-

Table 2. Optimum time and costs for crystal structure determinations. Costs, which will vary from institution to institution, are based on $50,000 per scientific man-year, $4 per kiloword hour on the CDC 6600 computer, and amortization of the diffractometer at $30,000 per year. The allocation of the diffractometer cost soars when the diffractometer is not in continuous use—a likely possibility in most laboratories. (a) A Caltech rapid analysis (29 atoms, one heavy) of Fig. 2b. (b) A 28-atom equal-atom structure (solved by direct methods) at Brookhaven. (c) A 55-atom heavy-atom structure at Brookhaven.

Problem	Man-months	Computer use (kiloword-hr)	Diffractometer time (weeks)	Costs (dollars)		
				Labor and overhead	Computer charges	Equipment amortization
a	½	150	1	2000	600	6000
b	1	200	2	4000	800	1200
c	2	1000	4	8000	4000	2500

tion $\mathbf{r}_j$, and a Debye-Waller factor T_j which describes the motion of the atom. The Fourier transform of $F(\mathbf{h})$ gives the scattering density of the crystal

$$\rho(\mathbf{r}) = \sum_{\mathbf{h}} F(\mathbf{h}) \exp\ (-2\pi i \mathbf{h} \cdot \mathbf{r}) \quad (3)$$

with peaks at the atomic positions (*6*).

The number of data—individual $F(\mathbf{h})$ measurements for different $\mathbf{h}$—required for a crystal structure determination may be estimated in two ways. For good resolution of atoms 1 Å apart, all reflections which can be collected with radiation with a wavelength of about 1.5 Å should be measured. This number is

$$N = (2/3)\pi(2/\lambda)^3 V_{\text{cell}} \approx 5 V_{\text{cell}} \quad (4)$$

where V_{cell} is the unit cell volume. The unit cells of small organic molecules have volumes of a few hundred cubic angstroms. Although symmetry may reduce the number of required data by factors which are often 2 or 4 but sometimes larger, this number will be a few thousand for most molecules of chemical interest. The average organic molecule will have a volume of about 10 Å^3 per atom, and the number of data required is thus seen to be about 50 per atom. For a rough structure determination, half this number may be adequate.

Another way of obtaining the estimate is to consider that it requires ten parameters to completely describe an atom: three positional coordinates, six amplitudes of thermal motion (the components of a symmetric second-order tensor), and a scale factor. For a sufficient degree of overdetermination in a least-squares refinement of the parameters, it is desirable to have five data points for each refined parameter. We again arrive at the estimate of 50 reflections per atom—two to five reflections per unit of molecular weight for organic compounds.

Diffraction Geometry

It is convenient in thinking about diffraction geometry to consider the reciprocal lattice—a geometrical array of points, with each of which is associated the Miller indices (h, k, l) of a diffracting plane. The conditions for diffraction are illustrated in Fig. 3. A sphere of radius $(1/\lambda)$ is imagined. This is called the Ewald sphere. The incident beam is directed toward the origin **0** of the reciprocal lattice. Diffraction occurs only when points of the reciprocal lattice lie on the sphere; the diffracted beam direction is given by the vector joining the center of the circle to the reciprocal lattice point. For a single position of the crystal, it may be seen that only a very few diffracted beams are produced. One is, however, free to give the crystal general three-dimensional rotations about the origin **0**. In so doing, he will cause many points of the reciprocal lattice to cut the sphere and hence produce x-ray diffraction maxima. All data collection methods may be discussed in terms of this concept.

Most of the earliest structure determinations by x-ray diffraction were carried out by the Braggs (*7*) who used a

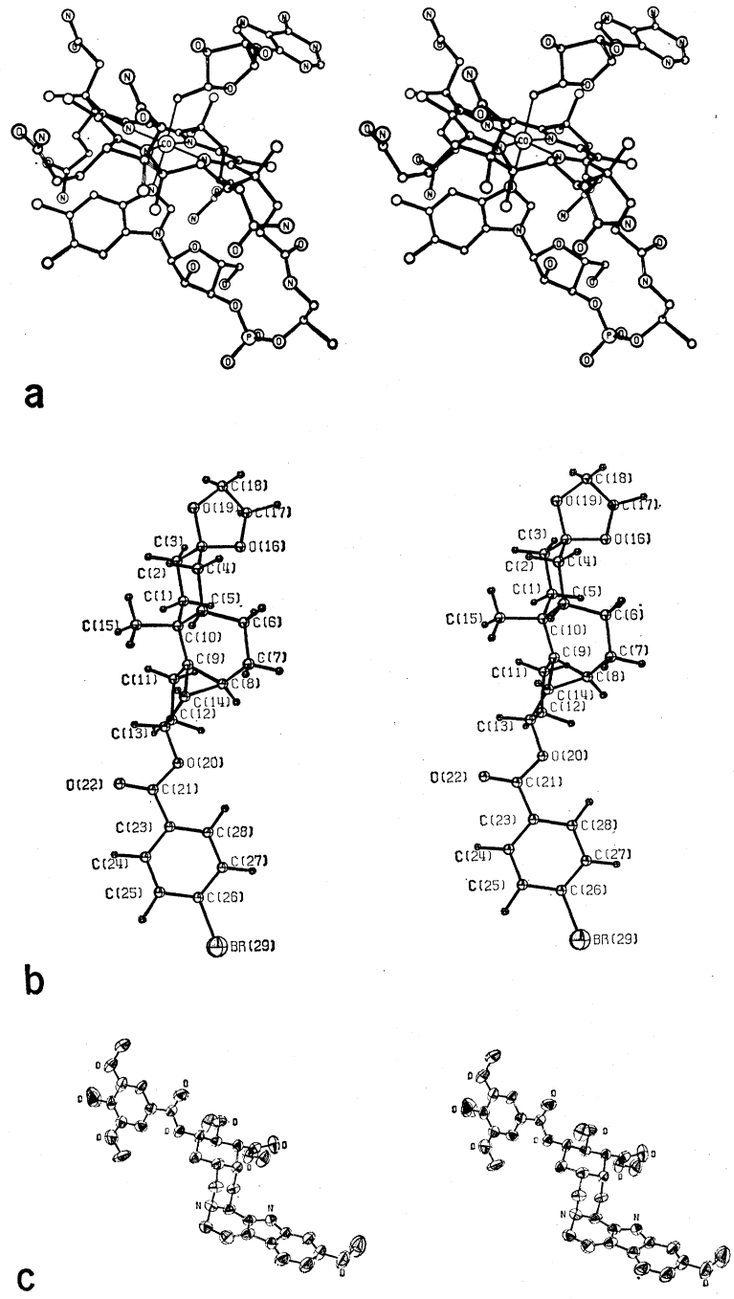

Fig. 2. Some stereoscopic drawings of crystal structures produced by a computer program (Ortep, 19). The drawings may be viewed by use of a small hand-held stereoscope or (by many people) without optical aids. (a) The structure of vitamin B_{12}, as determined by x-ray diffraction. (b) The structure of a carbene addition compound, $C_{24}H_{29}O_4Br$, determined in 10 days in the Caltech Rapid X-ray Analysis Project. (c) The structure of reserpine, $C_{33}H_{40}N_2O_9$, one of the largest acentric structures solved by direct methods.

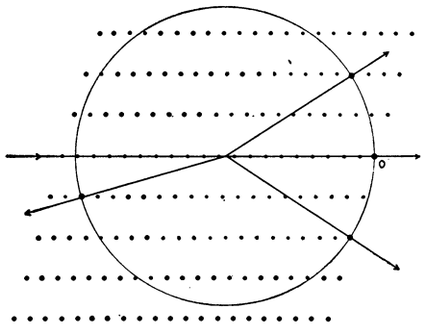

Fig. 3. Geometry of the diffraction experiment. A sphere with a radius of $1/\lambda$ is drawn, passing through the reciprocal lattice origin **0**. Diffraction occurs at special angles indicated by the arrows when reciprocal lattice points lie on the surface of the sphere. The angle between the incident beam passing through **0** and the diffracted beam is the scattering angle 2θ.

diffractometer. An ionization chamber placed in the direction of the diffracted beam was used to measure the diffracted radiation intensity. The crystal position, and hence the position of the reciprocal lattice relative to the Ewald sphere, was controlled by setting the positions of three Eulerian angles. For successive motions of the crystal and detector, the intensities of all possible reflections (h, k, l) could be measured. This is exactly the scheme used in most modern diffractometers, except that a single scintillation counter–photomultiplier detector is used (*8*).

It is clearly a tedious procedure to set the crystal orientation angles and the counter position manually, to initiate counting, and to record results for the thousands of Bragg reflections necessary for the solution of a moderately complex structure; however, a number of laboratories have been collecting data in this way for several years. The x-ray diffractometer was thus one of the earliest candidates for complete automatic control. Automatic control of the data collection procedure has been largely responsible for the revolution in crystallography.

Automatic Diffractometers

About 10 to 15 years ago, a number of laboratories and commercial firms developed automated diffractometers of the four-circle type and of types which simulated the geometry of various successful and traditional photographic data collection devices. The various angular settings of the diffractometer are controlled by motors; information for setting of the motor shafts to specific angles, for count initiation, and for data recording are entered into the control section of the diffractometer via punched cards or punched paper tape. The results are punched out on either of these media or possibly recorded on magnetic tape. The input data for the paper tape or cards are usually generated by a computer program which calculates the necessary information from the unit cell constants of the crystal, angles describing the initial orientation of the crystal, and parameters set by the experimenter for the particular run. The output data are read directly into a computer for processing and for structure solution.

Most x-ray diffractometers in use today operate in this way. Because of the automation, it is possible to collect routinely several hundred reflections per day on a round-the-clock basis. This means that a data set for a moderate-sized molecule may be obtained in less than a week. The scientist is freed for less routine duties, and there is less chance for human error in the data collection process. It is also worth noting that human error in the stage of setting up the experiment and determining the parameters for the run can be catastrophic, for in the automatic experiment there is no human there to recognize error when it occurs. Because the data collection process is rapid, many duplicate measurements may be made to give good estimates of data reproducibility. This has led to increased precision of crystal structure determinations.

Computer Control

The success of the automatic diffractometer as a routine laboratory instrument has been in large part due to the reliability and maintenance-free character of solid state logical circuitry used in the interface between input-output media and the mechanical and electrical devices to be controlled. The reliability and reduced cost of such circuitry has also led, in the past few years, to the development of inexpensive and powerful digital computers; such computers have now been widely adapted for diffractometer control (*9*, *10*). There are several advantages inherent in this development.

First of all, it is necessary for the experimenter to enter only a few parameters into the computer, which then calculates all the setting angles and other

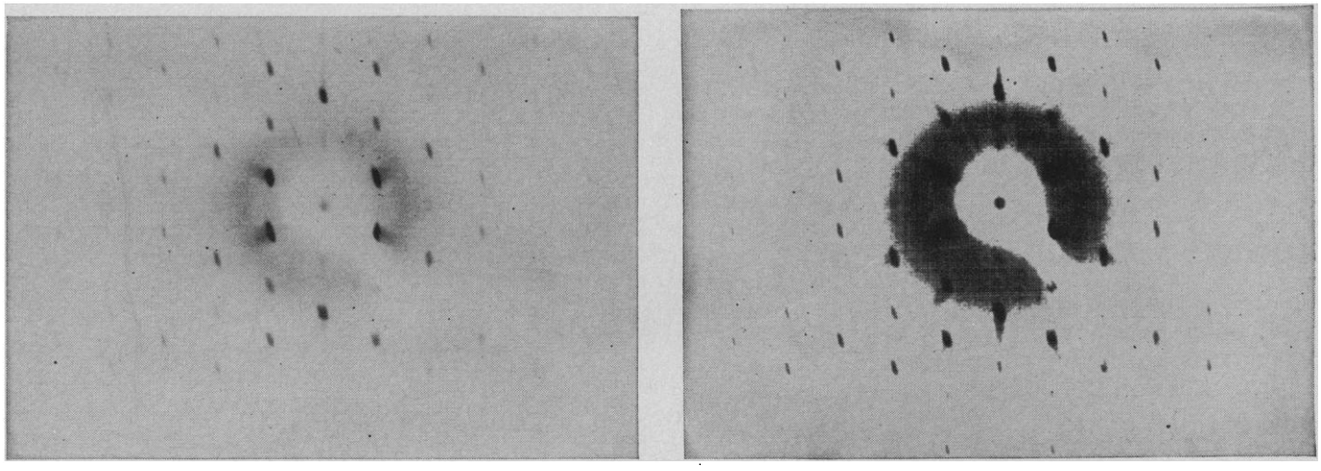

Fig. 4. Comparison of x-ray diffraction film and a computer-generated simulation of the same film in which the background has been suppressed. The original film was scanned by a scanning densitometer which recorded on magnetic tape the optical density at more than 1,000,000 points. The magnetic tape was processed through a computer, and the data was plotted on 35-mm film on a cathode ray tube (*11*).

Table 3. Number of reflections per 1° of crystal rotation for a position-sensitive detector or film. This number gives an approximate measure of the increased efficiency over that of a single counter. Data collected to a resolution of 1.54 Å. Adapted from Arndt (*12*).

Unit-cell edge (Å)	One-dimensional array	Two-dimensional array
10	0.4	3.2
20	1.5	26
50	9.7	412
60	14	690
100	39	3194

information necessary to take a piece of data. The large stacks of cards or rolls of paper tape with the inherent possibility of mechanical failure (card jams, tape tears) are eliminated. Second, in case the data-taking procedure is to be changed, the computer-controlled diffractometer will require only a change in a program—a software modification; the card- or tape-controlled diffractometer will require a change in wiring—a hardware modification. Thus much greater flexibility is available in the computer-controlled device. While watching data come out, the scientist may see a need for a change in the procedure for data collection. Often, the replacement of a single word in the computer memory may suffice to change the procedure immediately. A third advantage is that feedback is possible; the results of the experiment may be used to control future action. This is particularly valuable in monitoring the quality of the data and checking for stability in crystal reflectivity and orientation; it is also very valuable in the automatic determination of crystal orientation. A crystal may be put on a diffractometer, a computer program initiated, and, on the scientist's return from lunch, a set of orientation parameters may be printed out. A limited amount of data processing may also be done on-line: intensities may be corrected for background, and other correction factors may be applied to reduce the observed intensities to structure factors.

In many laboratories, another advantage of the computer-controlled diffractometer has been cost. When a computer adapted for time-sharing is available, the cost of the computer may be shared between a number of experiments. Each experiment may thus have a share in a much more powerful computer than any one experiment alone could have. For several years, nine diffractometers have been operating off the same computer at the Brookhaven National Laboratory high flux beam reactor (*9*). The cost of adding an x-ray diffractometer to this system was simply the cost of buying the mechanical equipment and $10,000 for the additional interface.

Coordinate Detectors

For most structural work, the computer-controlled automatic four-circle diffractometer has high enough efficiency, so that very little more is required. The necessary data for the structural solution of a moderate-sized molecule of unknown structure may be gathered in a week. This is not, however, the case for large molecules such as proteins. Not only is the number of data to be collected much larger, but there is also a problem of radiation damage to the crystal. A few hours in front of an intense x-radiation source may significantly affect the intensities of the Bragg reflections because of deterioration of the regularity of the crystal structure. The utmost efficiency is thus required in obtaining the most possible data for a given x-ray exposure. Examination of Fig. 3 reveals that for a large unit cell (close spacing of the points in the reciprocal lattice) many Bragg reflections will occur simultaneously or almost so; this is indeed true for proteins. Thus much interesting scattering that is not recorded by a single counter will occur. In general one needs counters *everywhere* surrounding the crystal.

The last statement is the basis for what has been the mainstay of crystallographic technique for many years—the use of photographic film as the quantum-counting medium. A piece of film is placed in the neighborhood of the crystal; this film is preferably wrapped around the crystal in the form of a cylinder. The film is exposed as the crystal slowly rotates in an x-ray beam. All radiation reflected by the crystal is received and recorded on the photographic film. Many types of elaborate cameras for such recording have been built. The photographic film is the simplest example of what may be called a *coordinate* or *position-sensitive detector*. The *intensity* and *position* of a diffraction maximum are simultaneously recorded.

Formerly, the intensities of the spots on the film were estimated by visual comparison with a standard intensity strip; this is again a slow and tedious

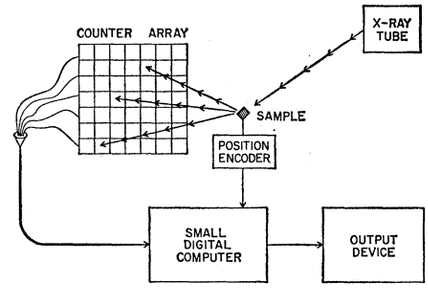

Fig. 5. A coordinate x-ray detector. The position of a detected x-ray quantum is recorded simultaneously with the crystal position. The results would usually be read directly into a digital computer for sorting and processing.

procedure. In more recent years intensities have been measured by the use of densitometers, and these have become increasingly automated. The film is automatically scanned, and the results are digitalized; the optical densities may be either transferred to magnetic tape or fed directly into a digital computer for processing. The results of such a digitalization of a photographic film are shown in Fig. 4 (*11*). A number of crystallographers whose specialty is proteins maintain that the use of film with automatic densitometric scanning is the preferred method of data collection from large unit-cell structures. Arndt has calculated the comparative efficiencies of film and single counters for unit cells of various sizes (*12*). The results, which clearly indicate the superiority of film for structures with large unit cells, are indicated in Table 3.

The fact remains that film is something of a nuisance. It requires processing; the linearity of response to x-rays is satisfactory only over a small range of intensities; and background and grain problems render the measurement of very weak reflections uncertain. Hence, the search is underway for methods which simulate film in its role as a coordinate detector but which do not have the disadvantages of film (Fig. 5).

One of the earliest proposed solutions to this problem was the use of the so-called spark chamber which has been successfully used in particle physics experiments (*13*). A grid of wires surrounded by an appropriate gas replaces the x-ray film. An x-ray quantum passing through the chamber will cause ionization of the gas, and, when a voltage is applied between the wire grid and ground, a discharge will take place. The location of the discharge may be identified by the potentials at the ends of the wires, thus leading to x and y coordinates for the event. Because of prob-

lems of efficiency and resolution, a successful x-ray diffractometer in which this principle is incorporated has not been built.

The availability of small, solid state detectors—sensitive with good resolution in the kilovolt x-ray range—has been the basis for another approach. An array of these small detectors may be built in any desired geometrical configuration, and the counts received in each detector may be processed in the usual way. This principle is being used in the diffractometer Aesop (*A*utomatic *E*qui-inclination *S*pectrometer *O*perating in *P*arallel) now being constructed by Dr. Robert Thomas and co-workers at the Brookhaven National Laboratory (*14*) (Fig. 6). A ring of 128 solid state detectors surrounds the crystal in a circular one-dimensional array. The crystal is rotated once, and all data from one plane of the reciprocal lattice are collected. The counting rate, angular position of the active counter, and crystal angular position are fed directly into a small digital computer which assigns Miller indices, derives structure factors, and records the data on magnetic tape for later use. Figure 6c presents a diagram which gives the angular position of the Bragg reflection plotted against the angle of crystal rotation. According to Table 3, the efficiency of Aesop should, for a crystal with a unit cell 60 by 60 by 60 Å (a protein four times the size of myoglobin), be about ten times more efficient than a single counter instrument.

The disadvantages of Aesop include the fact that there is a small dead space between the individual counters. This can be taken care of by the control computer: the diffractometer may be programmed to avoid these areas or some data may be taken twice. A more severe disadvantage is the expense involved in converting Aesop into a two-dimensional array necessary for the greatest efficiency in data collection (see Table 3). Something on the order of 10,000 individual counting units would be required.

The most successful approach to the two-dimensional array simulating a photographic film may be the use of a television camera as a recording device. This idea is being vigorously pursued by Arndt at the Medical Research Council in Cambridge as well as by groups in this country (*15*). The x-ray pattern is allowed to fall on a fluorescent screen rather than on a piece of film. Image intensification, focusing of the pattern on the face of a television camera tube, subsequent scanning of the tube, and digitalization of the results follow. The problems include those of sensitivity and linearity.

A promising technological advance is the silicon diode array television camera as used in the Picturephone (*16*). The conventional television image orthicon tube depends first upon conversion of the x-ray image to a visual image, which in turn is converted by the target of the tube into photoelectrons. X-rays generate charges directly on the silicon target tube; the charge image may then be scanned and digitized. The resolution possible in such tubes is entirely adequate for x-ray diffraction purposes, commercial tubes having diodes 8 μm in diameter spaced on 20-μm centers. The high resolution on a very small scale makes attractive the possibility of combining such detectors with tiny crystals and high intensity microfocus x-ray tubes. The imaging characteristics of the tube may also be used in the alignment stage.

In summary, the present automatic diffractometer can produce quantities of data sufficient for structure determination from crystals of moderate-sized molecules in less than 1 week. The new techniques as they continue to develop will greatly improve the experimental situation with regard to the collection of data from protein structures and will also allow complete data sets from smaller molecules to be collected in the matter of a few hours rather than a few days. This, coupled with the routine use of advanced computing techniques described in the following section, might reduce the time for a structural determination to the point where it may become even more a routine laboratory technique than it is today.

Impact of the Computer

The automation of data collection is half the reason for the revolution in crystallography, and the use of on-line digital computers in this automation has been discussed earlier. The other cause for the revolution may also be attributed to the computer: the use of the large-memory high-speed digital computer has been of the utmost importance in allowing rapid structure solution and parameter refinement.

The great barrier to routine crystal structure determination has always been the determination of the phases of the structure factors. These are complex quantities

$$\begin{aligned} F(\mathbf{h}) &= \sum_j f_j \exp\left[2\pi i(\mathbf{h}\cdot\mathbf{r}_j)\right] \\ &\equiv \sum_j f_j \cos 2\pi(\mathbf{h}\cdot\mathbf{r}_j) + \\ &\quad i\sum_j f_j \sin\left(2\pi\mathbf{h}\cdot\mathbf{r}_j\right) \qquad (5) \\ &\equiv A + iB = |F|e^{i\alpha} \end{aligned}$$

It is only the magnitude $|F|$ that is known. The phase factor α cannot be measured. In order to calculate a scattering density map by use of Eq. 3, the phases must be known. Fortunately, we know something about crystal structures which gives us some help in guessing the phases, although these guesses are far from obvious. The rather general conditions that the scattering density must be everywhere positive and that there are large zero areas between atoms lead to relationships that the phases must satisfy (*17*). The phases depend on these assumptions and on the magnitudes of the structure factors. The formulas that are derived for the phases are probability formulas and can lead to a satisfactory solution of a crystal structure only if a large number of accurately measured reflections are available. Here again the introduction of the automatic diffractometer has been important in supplying the large number of well-determined data. But more importantly, the application of the phase-determining formulas is a very tedious and time-consuming chore, even though the formulas are often mathematically simple. The combinatorial and logical manipulations involved have been successfully incorporated in a number of computer programs that can proceed more or less automatically from a set of input structure factor magnitudes to a set of phases.

In the case of centrosymmetric structures (structures where there is an atom at position $-\mathbf{r}$ for every atom at $\mathbf{r}$) the phases are all either 0 or π. Solution of such structures has become a very routine matter by the so-called direct methods, and structures with over 100 atoms in the molecule have been solved in this way (see Fig. 2c for an example). Structures that are not centrosymmetric have not as yet become quite so routine, but some with as many as 70 independent atoms have been solved.

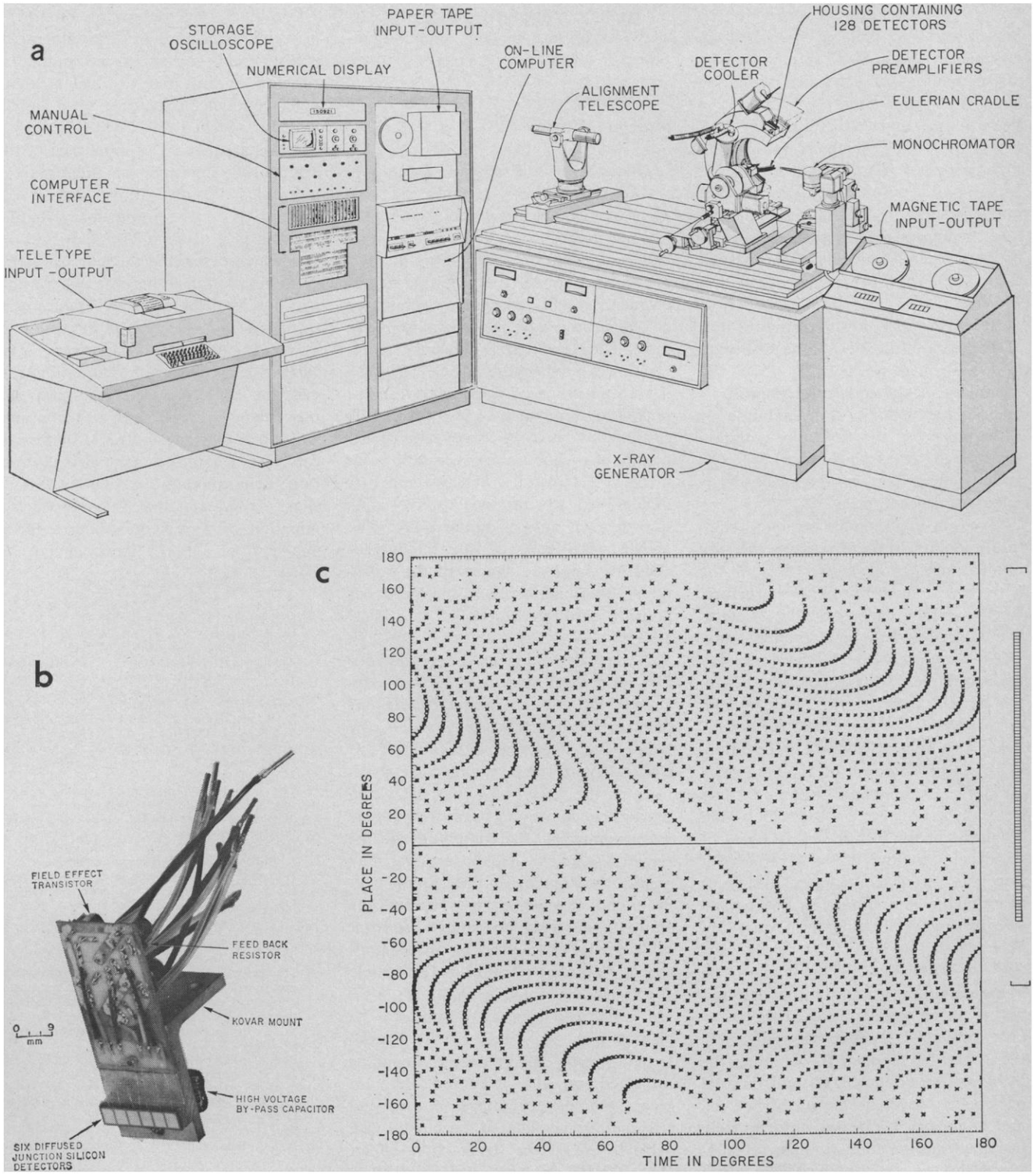

Fig. 6. (a) Drawing of a multicounter x-ray diffractometer—Aesop. The 128 silicon detectors are arranged in a ring surrounding the crystal and are maintained at liquid nitrogen temperature. (b) Silicon detectors used in Aesop. Six separate detectors etched on a single piece of silicon are shown, as are the preamplifiers. Twenty-two such pieces make up the entire detector array. (Photograph courtesy of Semiconductor Division of Edgerton, Germeshausen, and Grier). (c) Concept of Aesop. A given point on this diagram has two coordinates—time on the abscissa and scattering angle on the ordinate; these two coordinates may be correlated with two Miller indices—h and k if rotation is around the **c** axis of the crystal. The pattern generated is identical to that of the film produced in a Weissenberg goniometer, where the time (crystal rotation) coordinate is determined by a translation of the film along an axis parallel to the rotation axis of the crystal. The bar on the right represents the subdivision of the scattering angle by the 128 detectors. The experiment may be thought of as a movement of the bar across the diagram left to right.

The growing use of direct methods is indicated by the fact that, from a sample of structures reported in the September 1969 issue of *Acta Crystallographica*, 9 out of 27 were solved by direct methods of phase determination and the remainder by traditional heavy-atom (most structures) and Patterson methods. The figure for a comparable period in 1965 is 3 out of 27. In the past 2 years, the ratio of direct methods solutions to conventional solutions in papers read at the meetings of the American Crystallographic Association has increased from 1/4 to 1/1 (*18*).

In solving any crystal structure it is of course useful but not necessary to know as much as possible about the chemistry of the molecule. This information may help in the resolution of ambiguities in the structure solution step. Such constraints may even be automated as part of the phase-determination procedure.

Once an approximate structure solution is obtained, there remains the task of determining the best values for the parameters describing the structure. This is usually carried out by an iterative nonlinear least-squares procedure. For a structure with 50 atoms, we may wish to refine 450 parameters to obtain the best fit between the observed and calculated values for 2000 pieces of data. This is a formidable job and requires large amounts of time on even the largest computers. Running times for such a problem on the CDC 6600 would be about 20 minutes at a cost of $210 per iteration. It is this refinement step that often contributes the lion's share of the computing cost of a crystal structure determination; it is questionable whether it should always be carried to completion. If the aim of the investigation is primarily the determination of the configuration of the molecule and the connectivity diagram, there may be no point in obtaining the most precise bond lengths and thermal parameters—even if the quality of the data warrants it. It has unfortunately become traditional among many crystallographers to squeeze the last possible item of information out of their data even when this item of information may be basically uninteresting—or at least not worth its cost.

In any case, the large computer makes possible these refinements and the preparation of electron density maps evaluated at hundreds of thousands of points from thousands of pieces of data in a matter of minutes. These calculations—the heart of crystallographic refinement—are easily carried out on the modern digital computer (Table 2).

Structure Display

Once the crystal structure solution is accomplished, there remains the task of presenting the results to the chemist in a meaningful way. Three-dimensional ball and stick or space-filling models are the traditional tools of the stereochemist. Again, computer technology comes to the fore in extending these tools. The drawings of molecular structures presented in this article (Fig. 2) have been prepared by an elegant computer program which—given the atomic coordinates and thermal vibrational parameters—does the necessary geometrical calculations and graphic manipulations to produce stereoscopic drawings on an *x-y* plotter (*19*). The chemist can at once see the stereochemistry and the shapes of the vibrational ellipsoids without the need for pouring through masses of numerical tables.

A new development in graphic display of crystal structures is that associated with on-line interactive display devices. In a system developed at the Brookhaven National Laboratory (*20*) we are able to display molecular structures on the screen of a color television tube. The coordinates for the display are generated by the computer, and the scientist seated at a data terminal may modify the drawing on-line. Atoms may be added, deleted, or moved, and the entire model may be rotated in front of the viewer's eyes. In the stereoscopic pictures presented in this article a left-eye view and a right-eye view are presented separately—one to be viewed with each eye. In the Brookhaven system, the right-eye image is projected on the television tube in green, the left-eye image in red, and red and green filtered spectacles are worn to obtain the stereoscopic effect. One is able to work rapidly with a three-dimensional model.

This tool can be of great use in the structure determination step: trial structures may be manipulated and the reasonableness of intermolecular contacts can be ascertained. The interaction feature between man and computer is very important and has been of great usefulness in the building of models of large biological structures in similar systems (*21*).

Although the determination of a molecular structure by crystallography will probably never be as routine as running an infrared or nuclear magnetic resonance spectrum of a liquid, we have reached the day when such a determination is an essential part of the arsenal of any chemist interested in molecular configuration—and what chemist is not? Automatic structure solution is a reality (*22*), and the time and expense for a molecular geometry determination have become comparable with many of the other techniques used in chemical research. The development of more efficient diffractometers, the further development of automatic methods for structure solution, and improvement in crystal growth techniques will mean that any molecule that exists can have a complete geometrical structure determination carried out in a short period of time. Even the determination of a protein structure forms only part of a Ph.D. thesis now (*23*).

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Inborn Errors of Mucopolysaccharide Metabolism

Faulty degradative mechanisms are implicated in this group of human diseases.

Elizabeth F. Neufeld and Joseph C. Fratantoni

Inherited metabolic diseases generate research activity of far greater intensity than one might expect from their relatively rare occurrence. This is because genetic disorders afford a unique opportunity to combine the concepts of genetics with the tools of biochemistry to study the metabolism of man, as has been so successfully done for the metabolism of microorganisms. The lesson of genetics is clear: genes contain the code for the structure of proteins; a mutation in a gene will result in an alteration of the specific protein to which that gene holds the code. The result may be benign or disastrous, depending on the importance of the protein to the overall metabolism and on the effect of the structural change on its function. Faced with a disease of genetic origin, the biochemist's task is to identify the altered protein which is specific to that disorder. Success may lead both to practical applications in the management of the disease and to a clearer understanding of normal metabolic processes.

Mucopolysaccharide Disorders

The best known and most severe of the inherited disorders of mucopolysaccharide metabolism is the Hurler syndrome, named after the pediatrician, Gertrud Hurler, who described it in great detail in 1919 (*1*, *2*). After several months of normal development, the infant deteriorates physically and mentally and gradually acquires an extraordinary appearance. The head is large with a flat bridge of the nose, wide-set eyes, large lips, and coarse tongue. The nasal deformity causes obstruction to breathing and may be the first abnormality noted by the parents. Growth is stunted, corneas become cloudy, and hearing deteriorates. There is widespread skeletal involvement, with stiff joints, widened ribs, and aberrant development of the vertebrae and long bones. The liver and spleen are greatly enlarged. Abnormalities are found in the walls of the major blood vessels and in the heart valves, leading to cardiovascular complications. Mental retardation is prominent, the brain suffering damage both from cellular defects and from the hydrocephalus due to impaired cerebrospinal fluid mechanics. Affected children usually do not survive through the second decade.

A closely related disorder, the Hunter syndrome, follows a milder course (*2*). Occasionally, an affected individual may live well into adulthood. The corneas remain clear, and mental retardation is variable; of the two brothers described in the original report by Charles Hunter, in 1917, one was bright (*3*).

A major difference between the Hurler and Hunter syndromes is in the mode of inheritance. The Hurler syndrome is transmitted in classical Mendelian fashion as an autosomal recessive; it can occur in children of either sex whose parents, though carriers of the Hurler gene, show no apparent abnormality. The Hunter syndrome is sex-linked, like hemophilia. Women who are carriers can transmit the disease to their sons but not to their daughters; half the daughters, however, are likely to be carriers and in turn transmit the disease to their sons.

Yet another disorder, the Sanfilippo syndrome, resembles both the Hurler and Hunter syndromes, except that the physical defects are relatively mild while mental retardation is severe. It is transmitted, like the Hurler syndrome, as an autosomal recessive. Originally thought to be a "forme fruste" (that is, an incompletely expressed form) of the Hurler syndrome, it became recognized as a separate disease entity in the early 1960's (*4*).

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