ELECTRON DIFFRACTION TECHNIQUE FOR THE DETERMINATION OF CRYSTALLINE STRUCTURE OF BETA GLYCINE

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(Ph. D. Thesis)

October 1970

AEC Contract No. W-7405-eng-48

LAWRENCE RADIATION LABORATORY
UNIVERSITY of CALIFORNIA BERKELEY
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ELECTRON DIFFRACTION TECHNIQUE FOR THE DETERMINATION
OF THE CRYSTALLINE STRUCTURE OF β GLYCINE

By

Wai-Kit Quon

ABSTRACT

The method of electron diffraction is applied to the investigation of the crystalline structure of β glycine. The structure as determined by x-ray diffraction methods is used as a standard of the investigation. The emphasis of the research is therefore on the evaluation of the electron diffraction technique and the effects of dynamical scattering, rather than on the redetermination of the glycine structure.

It is shown that the Patterson map is similar to the inter-atomic vector map calculated from the atom coordinates of the x-ray determined standard structure. Hence it can either be interpreted kinematically, or it is not sensitive enough to show moderate dynamical effects.

A test based on the Two Beam dynamical scattering theory shows that the sample is weakly dynamical and has an estimated mean thickness of about 1300 Å.

Dynamical effects play a more important role in electron diffraction than in x-ray diffraction. The effect of dynamical scattering is not corrected for in the work reported here, and it is found that the resulting reliability
factor of the determined structure is about 0.48, which indicates a very low level of confidence. Therefore, dynamical scattering must be corrected for if an accurate determination of crystal structure is to be attempted.

Hydrogen positions are easily detectable in the difference Fourier synthesis map. This shows that scattering by hydrogen is much stronger in electron diffraction than in x-ray diffraction. It is suggested that hydrogen atoms should be included into the structural analysis at an earlier stage than that of the normal x-ray diffraction procedures.

The extent to which dynamical scattering limits the usefulness of electron diffraction intensity data for crystallographic determination has also evident significance for the interpretation of high resolution structures that might be obtained by electron optical imaging of the scattered radiation.
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PART I:

INTRODUCTION AND THEORY
I. INTRODUCTION

The phenomenon of electron diffraction was first discovered in 1927 by Davison and Germer. The first photographic record of diffraction patterns was obtained by G. P. Thomson from polycrystalline films of gold and other metals [1]. Since their discovery, electron diffraction methods have constituted an important tool in solving the structure of matter, in addition to or supplementing the method of x-ray diffraction, which was first demonstrated in 1912 [2,5,4]. X-ray diffraction has of course proven to be the more powerful tool of the two in the structural determination of crystalline materials.

Electron, x-ray and neutron diffraction share the same basic scattering theory. X-ray diffraction gives the distribution of electron density, electron diffraction gives the distribution of electric potential and neutron diffraction gives the distribution of nuclear scattering potentials within the crystal. Any of these distributions, however, is able to map the locations of atoms within the molecule. All three methods compliment each other. X-ray diffraction leads substantially in both the degree of reliability and sophistication, while electron diffraction and neutron diffraction
have yet to come of age.

While x-ray diffraction has many advantages over the other techniques, electron diffraction can claim the following advantages which makes it desirable:

1. The ability to handle extremely small quantities of material (about $10^{-12}$ gm quite easily). X-ray diffraction in general requires considerably larger crystals to be grown.
2. Simplicity in specimen preparation.
3. The ability to observe visually the diffraction pattern before recording.
4. The intensities of reflection are much higher than in either x-ray or neutron diffraction ($10^6$ times higher than for x-rays and $10^8$ times higher than for neutron [5]). This enables much shorter recording times.
5. With selected area diffraction techniques, an image of the specimen area under investigation can be obtained in conjunction with the recording of a diffraction pattern. Radiation damage may be the ultimate limitation to the amount of useful information that can be gotten from either diffraction or transmission data [6].

Conversely, the disadvantages at the present time are:

1. Operation in vacuum where certain crystalline structure might be disordered. Examples are specimens that must be hydrated.
2. Difficulty in determining the crystal orientation in
the preparation of specimens. This poses great problems in indexing a new structure.

3. Relative to x-ray, the interaction of electrons with matter is much stronger. While this feature ensures small specimen volumes and short exposure times it also limits the thickness of a specimen that can be used. Dynamical scattering effects are much more easily encountered [7].

The special characteristics of electron diffraction that are mentioned above makes it potentially an extremely useful tool in the investigation of biological specimens such as proteins and membrane structures because of inherently small specimen size in most cases. Part of the purpose of the work reported here is to investigate in a practical sort of way the extent to which dynamical scattering would limit the usefulness of electron diffraction intensity data for crystallographic work. The work reported here has also some evident significance for the interpretation of high resolution structures that might be obtained by electron optical imaging of the scattered radiation.

The other difficulties of specimen hydration, specimen orientation, indexing are largely of an instrumental and technical nature, and can presumably be overcome without great difficulty.

Most efforts in the application of electron diffraction have been in the studies of inorganic material [8,9]. The
application to organic materials such as the determination of the structures of paraffin by Vainshtein et al. [10], cis-dichloroethylene by Hoffman [11] and perfluorotrtrimethylamine and trifluoroethanol by Vaughn [12], are relatively infrequent. As far as biological structures are concerned electron diffraction is a relatively new tool [9, 13, 14, 15]. An optical diffraction technique which uses an electron micrograph of the specimen was developed by Klug and Berger [16]. Methods of reconstructing the three dimensional structure using the Fourier projection theorem have been applied to biological structures with helical and ocosahedral symmetry by Klug, DeRosier and others [17, 18, 19, 20]. Direct structural determination of biologically significant molecules is rare. An example is diketopiperazine by Vainshtein [21]. Diketopiperazine groups play an important role in the structure of proteins.

There has been no work done previously on the determination of the crystalline structures of amino acids, which have been accurately determined by x-ray, however [22].

This research sets out to investigate one of the simplest of the biologically relevant molecules, the amino acid glycine. The reason of the choice is that glycine is the smallest and simplest amino acid. Not including hydrogen atoms it has only five atoms in the molecule. This fact greatly reduces the number of parameters to be determined in the structural programs. It is very common and can easily be crystallized.
Its structure has been very accurately determined by x-ray diffraction [23, 24, 25]. The structure of beta glycine as determined by Iitaka [24] is used both as a guide as well as a standard for this investigation.

The main emphasis of this work has not been on the refinement and achievement of absolute accuracy in determining the bond lengths and bond angles, as would be the case for normal crystallography. Rather, this thesis shows the possibility and a set of procedures to do so for future work. This investigation must be viewed as a preliminary study of the application of the technique of electron diffraction to the determination of structure of amino acids and other biologically relevant structures.

This research responds to the following objectives:

1. A critical evaluation of the experimental procedure.
2. The extent of dynamical scattering.
3. Empirically testing the sensitivity of the Patterson function to the existence of dynamical effects in the diffraction intensities.
4. The characteristics of the x-ray structural determination computer programs after being adapted for electron diffraction uses.
   a. To modify programs for electron diffraction work.
   b. To test convergence of Least Square method using typical electron diffraction data.
II. THEORY OF ELECTRON DIFFRACTION

A. Kinematical Approximation

The motion of electrons is described by the Schroedinger wave equation,

$$\psi^2 \psi + \left(8\pi^2 m / h^2 \right) (E-V) \psi = 0 \quad (201)$$

where $\psi$ is the wave function, $m$ is the mass of the electron, $h$ is the Planck's constant, $E$ is the total energy of the electron, and $V$ is the potential energy of the matter through which the electron passes. $V$ must be equated to

$$V = e\psi \quad (202)$$

where $\psi$ is the scattering potential within the crystal and $e$ is the charge of one electron. The electron beam incident upon the surface of the crystal may be described by a plane monochromatic wave

$$\psi_0 = A \exp(ik_0 x) \quad (203)$$

where $A$ is the amplitude and $k_0 / 2\pi = 1 / \lambda = (2mE/R)^{1/2}$ and $x$ is the generalized coordinate. $\psi_0$ is the solution to eq. 201 when $V = 0$.

For a kinematic approximation [26], [27], [28], the problem of the electron scattering by matter may be solved by the first order perturbation method [29]. This method assumes
that \( \Psi \) is composed of two terms, the wave function \( \Psi_0 \) and a scattered wave \( \Psi' \).

\[
\Psi = \Psi_0 + \Psi'
\]

The substitution of eq. 204 into 201 gives

\[
\nabla^2 \Psi + k_0^2 \Psi = U(r)(\Psi_0 + \Psi')
\]

where

\[
U(r) = (8\pi^2 me/h^2) \psi(r)
\]

Eq. 205 is the general equation of the form

\[
\nabla^2 \Psi' + k_0^2 \Psi = U(r) \psi(r)
\]

the solution of which [30], is

\[
\Psi_k(r) = -\frac{1}{4\pi} \int U(r_1) \psi(r_1) \frac{\exp(ikR)}{R} r_1
\]

where \( R = |r-r_1| \) and \( r_1 \) is a vector within the scattering volume \( v \). Since \( \Psi = \Psi_0 t \psi' \), the required solution is actually an integral for \( \psi(r_1) \).

\[
\psi'(r) = -\frac{1}{4\pi} \int U(r_1) [\psi_0(r_1) + \psi'(r_1)] \frac{\exp(ikR)}{R} r_1
\]

Eq. 209 states that the scattered waves are generated by waves travelling in the scattering field \( U(r_1) \) of the sample, \( \psi_0 \) being the initial wave and \( \psi' \) the scattered wave.

The kinematic approximation or the first Born approximation assumes that the scattered waves are weak compared with the initial wave,

\[
\psi' \ll \psi_0
\]

This means that only the initial wave gives rise to new
scattered waves and that the term $U \cdot \psi'$ representing the scattering of secondary waves is negligible. Therefore the term $\psi_0^* + \psi'$ in Eq. 209 can be replaced by $\psi_0 = A \exp(ik \cdot r)$.

Eq. 209 is then approximated to be

$$\psi'(\hat{r}) = \frac{1}{4\pi} \int U(\hat{r}_1) \psi_0(\hat{r}_1) \frac{\exp(ikR)}{R} \, d\hat{r}_1,$$

which can be shown [30] to be

$$\psi_1(\hat{r}) = -\frac{1}{4\pi} \phi(\hat{s}) \frac{A \exp(ikr)}{r},$$

where

$$\phi(\hat{s}) = \frac{2\pi me}{\hbar^2} \int \psi(\hat{r}) \exp[ifs \cdot \hat{r}] \, d\hat{r}_1.$$  

The scattered wave is then shown by Eq. 212 to be a spherical wave with an amplitude proportional to the Fourier integral over the potential of the object, Eq. 213 and Eq. 212 are valid when $\psi_1(\hat{r})$ is viewed at a great distance from the object.

The kinematic theory of electron scattering assumes the validity of the Born approximation, which is not used in the dynamical theory, since the dynamical theory takes into account the interference of both the primary and the secondary waves, which produces a complex wave field in the crystal. This complex wave field makes the scattering potential a complex function rather than a real function.

The flow of particles through unit area, $J$, is determined by

$$J = \frac{(h/4\pi m)(\psi^* \nabla \psi - \psi \nabla \psi^*)}.$$

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Thus, the substitution of $\psi_1$ gives

$$J_r = (J_0/r^2)|\phi(s)|^2,$$

where $J_0$ is the intensity of the incident beam. It should be noted that $r$ is the distance from the specimen to the screen and is a constant. Therefore, Eq. 215 goes into

$$I_r|\phi|^2$$

which defines the important relationship between the relative scattered intensity and the structural amplitude, which in turn relates $I_{hkl}$ to the real structure, $\psi(\vec{r})$.

Eq. 213 shows that the structure amplitude is the inverse Fourier transform of the scattering potential of the crystal. This potential is the potential of the unit cell. If this is approximated by a superposition of the potential for each of the atoms within the unit cell

$$\psi(\vec{r}) = \sum \psi_i(\vec{r} - \vec{r}_i)$$

then upon substituting into Eq. 213, we find that the structure amplitude can be expressed as the summation of Fourier integrals over atoms, with a phase factor $\exp[U(\vec{s} \cdot \vec{r}_i)]$

$$\phi_{hkl} = \sum f_{el_i} \exp[2\pi i(\vec{r}_i \cdot \vec{H})]$$

where $\vec{H} = \vec{s}/2\pi$. In this equation $f_{el_i}$ is the scattering factor of atom $i$ and $\vec{H}$ is the reciprocal lattice vector. A table of the atomic scattering factors for H, C, N, and O is listed in Appendix A.

The atom scattering factor $f_{el}$ must be modified by the
temperature function \( f_T \) to take into account the thermal vibrations of the atoms.

\[
f_{e1,T} = f_{e1} \cdot f_T
\]

where \( f_T = \exp[-B(\sin\theta/\lambda)^2] \)

\( B \) is the temperature factor.

Thus, given the atom coordinates and the atomic scattering factor, the scattering amplitude \( \phi_{hkl} \) can be calculated by Eq. 218. On the other hand, given the scattering amplitude (from experiment, by measuring \( I_{hkl} \)) and if the phases are also known an inverse Fourier transform of Eq. 218 gives the distribution of scattering potential within the unit cell.

Since \( \phi = F^{-1}(\psi) \)

then \( \psi = F(\phi) \)

where \( F \) represents the Fourier operator and \( F^{-1} \) the inverse Fourier operator.

**B. The Patterson Function**

If Eq. 222 is calculated with \( |\phi|^2 \) instead of \( \phi \), then it can sidestep the phase problem since by squaring the scattering amplitude, the phase term is equal to unity. This is the same as doing the Fourier summation over the diffraction intensity \( I_{hkl} \) as given by Eq. 216. The Fourier integral over the intensity of the diffraction pattern is called the Patterson function or

\[
p = F(\phi^2)
\]
Because Eq. 221 shows that $\phi$ itself is an inverse Fourier transform of $\psi$, the Patterson function can be interpreted as

$$P = F[F^{-1}(\psi) \cdot F^*^{-1}(\psi)]$$

or

$$P = \psi \ast \psi$$

where the symbol $\ast$ denotes the convolution operation. The Patterson function is thus seen to be the autocorrelation of the scattering potential. The Patterson function defined by Eq. 224 will show peaks for values of functional displacements which produce superposition of scattering centers. Hence, a Patterson function plot results in a plot of the interatomic vectors within the unit cell.

The Patterson function, when interpreted kinematically, can be used to find scattering centers, i.e., atoms, within the unit cell, if the number of atoms is small. If the number of atoms within the unit cell is large (above 10 for example) interpretation becomes virtually impossible [31],[32].

C. Dynamical Scattering

Most of the work done so far on the diffraction of either x-rays, neutrons, or electrons as a tool for structural determination has been based on the kinematical theory because it has a wide range of validity over most experimental conditions. Treatments based upon the dynamical theory are at best complicated exercises because a practical theory governing the range of dynamical scattering is still in want.
A dynamical approximation which has been widely applied was originally proposed by Blackman [33]. It is based on the two beam model, in which it is assumed that one scattered secondary beam besides the central beam can give rise to secondary scattering. For the case of a crystal of "medium" thickness with a weak dynamical effect, the relative intensity of diffraction can be shown [3] to be

\[ I_{nk\ell} = \lambda^2 |\frac{\Phi_{hk\ell}}{\Omega}|^2 \cdot t \cdot K(t, \phi) \]

where \( \Omega \) is the unit cell volume, \( t \) the thickness of the crystal and \( K \) a correction factor of the form

\[ K(t, \phi) \sim \exp\left(-\frac{1}{3}(t\lambda|\frac{\Phi_{hk\ell}}{\Omega}|)^2\right) \]

Schiff [44] shows another way of treating dynamical scattering. Assuming an infinite order of multiple scattering, he arrives at the formulation

\[ \Phi \sim F(1-e^{-i\psi'}) \]

where \( \psi' \) is the two dimensional projection of the scattering potential. This approximation is valid only at small scattering angles. That is, if the Ewald sphere is perfectly flat so that the following equation applies

\[ \psi'(x,y) = \int \psi(x,y,z) \delta z \]

Cowley and Moody [34] use an approximation which corresponds to a successive increase in the number of two dimensional distribution of potentials (Eq. 228) by which the three dimensional field is approximated. If the number of
sections is one, they arrive at a pseudokinematic theory

\[ \Phi \sim \exp(i\omega') \]

with \[ \sigma = \frac{2\pi m\lambda}{\hbar^2} \]

In the special case of a two beam approximation, they give essentially the same result as the dynamical theory of Bethe [45].

The Patterson function is difficult to interpret with the dynamical formulation, but it can be shown (9 and Ch. VIII) that it might not differ significantly from the Patterson function calculated from the kinematical model for the cases of light atoms if the dynamical effect is not too strong. This point is discussed at greater length in Chapter VIII.
PART II:

METHOD AND MATERIAL
III. SPECIMEN PREPARATION

Three polymorphic forms of glycine have been reported: (1) an ordinary form, alpha. The crystal structure was first determined by Albrecht and Corey [35] in 1939. It was reetermined very precisely by Marsh [23] in 1958. (2) An unstable form, beta. It was first described by Fischer [36] in 1905. Iitaka [24] in 1959 determined its structure by x-ray diffraction and showed that single molecular layers, whose internal arrangement is the same as in the alpha form, are held together by hydrogen bonds throughout the crystal. (3) A third form, gamma, is strongly piezoelectric and crystallizes with trigonal hemihedral symmetry. Its structure was determined by Iitaka [25] in 1959.

Iitaka reports that beta glycine crystallizes in the form of small needles and can be prepared by adding ethyl alcohol to a concentrated aqueous solution of glycine. He also reports that beta glycine readily transforms into the alpha form in air, but remains unchanged indefinitely if kept in a dry atmosphere.

However, in our experiments, beta glycine is readily crystallized by evaporating in a 60°C oven small droplets of half concentrated aqueous solution, without adding ethyl alcohol. The micro-crystals thus produced remain as far as
we can determine, indefinitely without changing into the alpha form.

Our experimental procedure is as follows: 200 mesh copper electron microscope sample holders are used. On top of the sample holder is deposited a thin film of formvar to serve as the specimen support. A small droplet of about half concentrated solution of glycine in distilled water is deposited on top of the formvar film. The sample holder is then put for a few minutes inside a dry oven heated constantly at 60° C. Micro-crystals of glycine are readily formed on the formvar film.

A thin carbon film is then evaporated in vacuum onto the specimen. This is done so that the specimen is more stable in the electron beam of the electron microscope, and to avoid electrical charging of the specimen during electron irradiation.

Specimens prepared in this way have the disadvantage that probably all three crystalline forms will be present. There is no physical way to separate them. The diffraction pattern of this specimen shows predominantly three different types of spot patterns. These have been indexed to be:

a. The hkO plane of β glycine
b. The h01 plane of β glycine
c. The hko plane of alpha glycine

Among the three spot patterns, the hkO plane of β glycine is the most frequent and most stable. Next in frequency
of appearance is the hkO plane of alpha glycine, while that of hOl of 6 glycine is relatively rare.

Spot patterns of other planes are not encountered, neither did we find any pattern of the gamma form. The frequent appearance of the hkO planes agree with the fact that the C axis is the axis of growth of crystals, and the crystal grows with its elongated axis perpendicular to the specimen support. The fact that spot patterns rather than rings are formed indicates that the crystals on the average are longer than the effective diameter of the electron beam.

The hkO plane spot pattern of alpha glycine is often incomplete and warped. This is probably due to a poor mosaic structure of these crystals.

The diffraction patterns of 6 glycine, on the other hand, are quite stable. Both the hkO and the hOl planes are clear and without distortion. They both have an estimated fading time of about three minutes at 80 kv, under the conditions of illumination used in this work.

The 6 glycine patterns, both hkO and hOl are shown in Figure 602 and 603 respectively.
IV. SELECTED AREA DIFFRACTION

Selected Area diffraction techniques [13] and [28] are specially suited for choosing single particles, single crystals, or small portions of a specimen for diffraction patterns. Normally the procedure is that an area is selected from the image on the fluorescent screen. The optical mode of the column is then changed to present the diffraction pattern, which corresponds to the area selected from the image in the magnification mode. A diffraction spot pattern is formed if the specimen is crystalline.

After an area has been selected, the column is switched to the Selected Area Diffraction (SAD) mode. The SAD mode leaves the illumination system and the objective lens unchanged, but the intermediate lens is changed in focal length so that it has a new object plane (Figure 401). This new object is the back focal plane of the unchanged objective lens, instead of the image plane of the objective lens as was the case with normal microscopy.

At the back focal plane of the objective lens, for parallel illumination entering the lens, there is by definition a focused image of the source. If a crystalline specimen is placed in the object plane of the lens (objective), a diffraction pattern will be formed at the back focal plane.
Figure 401

Ray diagram of the selected area diffraction mode of operation of the electron microscope.
Figure 401.
By using the back focal plane of the objective lens as the object plane for the intermediate lens, the remaining lenses of the column magnify and transfer the diffraction pattern to the fluorescent screen.

In the SAD mode, the primary diffraction camera length is the focal length of the objective lens which is about 3-4 mm. The magnification introduced by the post-objective lenses, i.e., the intermediate and projector, in transferring the pattern to the screen, makes an effective camera length of the order of 40-50 cm.

In doing SAD diffraction, a real image of the specimen is formed in a plane close to the intermediate lens. The diffraction aperture (of the field limited aperture, as it is also called) placed in this plane will define the area of the specimen contributing to the diffraction pattern. Since the magnification of the objective lens is about 40x, a 25μ diffraction aperture will define an area of 0.6μm at the specimen[13].

Agar [37] discussed some precautions which must be taken if the area selection must be extremely precise.

In actual experiments, it was found that the specimen is extremely sensitive to the electron beam. It was estimated that the glycine specimen has a fading time of about 3 minutes. Therefore, the following practices were used:

(1) Keeping the filament current and bias to the lowest minimum.

(2) Use the smallest condensor aperture.
(3) Do not go through the magnification mode, i.e., as soon as the sample is mounted, operate in the SAD mode immediately. This is possible because there are enough thin crystalline areas to give ample diffraction patterns in a single specimen grid preparation.

(4) Any one sample is never used twice.

After a sufficiently clear diffraction pattern is found, a photograph is immediately taken. The usual exposure time is 3 minutes.
V. DATA COLLECTION

The presently used data collection system is as outlined in Figure 501. The procedure is as follows:

1. Electron diffraction patterns are recorded by photographic plates which are then developed.

2. The plate is then scanned by the Joyce microdensitometer. A tracing of each row or column is produced.

3. The response of the photographic plate to the electron beam and the output of the microdensitometer are calibrated. The systematic error due to non-linearity can be approximated empirically by assuming an overall exponential correction function.

4. The background is subtracted by visually drawing onto the microdensitometer tracing a background line and reading off from it a net density.

5. Data from the microdensitometer tracing are then corrected for non-linearity and crystal angular distribution by a PDP-81 computer.

6. The corrected, indexed data are then punched into IBM cards, which serve as the input to the program INCOR. INCOR is the first of the structural determination programs. It is not difficult to see that the data collection system as it is set up now is ill-equipped to handle large amounts in a
Figure 5.01

Data collection and conversion system.
ELECTRON MICROSCOPE DIFFRACTION PLATES (A.E.I.)

Output

MICRODENSITOMETER TRACING (Joyce)

Output

CORRECTION NON-LINEARITY Etc. (PDP8-I)

Densitometer tracing

CORRECTED digitized data

INCOR (CDC6600)

TAPE 5

Figure 501.
speedy manner, which is absolutely necessary if accurate determination of crystal structure is to be carried out. Compared with the levels of sophistication that x-ray crystallography has developed, this kind of experimental setup is still in its infancy.

Conceptually it is an easy extension to completely automate the process which was outlined in Figure 5.1. Such an attempt is now being developed in our laboratory. The automated system would involve a TV image intensifier and a video disc recorder to take the place of the camera plate. Signals from the video recorder will go through an analog to digital converter unit. The digitized data will be indexed and corrected for non-linearity of calibration, etc., with the help of a computer. The output from this should be repacked and written into magnetic tape to be used as the input tape of structure determination programs.
VI. DATA CONVERSION

A. Indexing

1. Calibration of the Camera Length

The effective camera length of the electron microscope is calibrated with a gold powder pattern. The d₁₁₁ and d₂₀₀ rings of the powder are used as standard, since they are known to have the distances 2.356 Å and 2.04 Å respectively. A selected area diffraction pattern superimposed on the selected area image is shown in Figure 601.

2. Indexing of the h₀k₀ Pattern

The unit cell dimensions are measured with the equation

\[ d = \frac{\lambda L}{r} \]  

where \( \lambda \) is the wavelength, \( L \) the effective camera length and \( r \) the distance from the (000) central spot. The result is shown in Table 601. The estimated error of the measurement is less than 2%. In the diffraction pattern along the 0k0 row, the symmetry-forbidden reflections for \( k \) equals to odd integers do appear. If the space group and unit cell dimensions were not previously known, this would present difficulties in indexing. The significance of the appearing of these systematic absences are discussed in Ch. X. The angle between
Figure 601

Selected area diffraction pattern of gold powder. The diffraction pattern is superimposed on the selected area image.
the 0k0 and h00 axes is 90°.

3. Indexing of the h0l Pattern

According to the known space group P$_{21}$ [38] there are no systematic absences in this projection. The angle between the h00 and the 00l axes is measured to be 67.0° which is the angle $\beta$ in the reciprocal space. Figure 603 shows the h0l projection.

The above measurements confirm the space group of P$_{21}$ as given by Iitaka [24].

B. Calibration

The intensities of electron diffraction spots vary tremendously, sometimes differing by as much as several orders of magnitude. One way of calibrating the photographic response over this range of intensities is by internal calibration. This is done by doing multiple exposures of the same pattern and by noting the differences in exposure times and the changes in photographic blackening [39, 40]. In this way, a blackening curve is constructed every time. This is the best way of calibration if not the most convenient one.

It was mentioned before that glycine crystals have a fading time of about three minutes under the present experimental conditions. This creates a problem for internal calibration, because the structure becomes disordered after several exposures. If one does multiple exposure with
Table 601
Experimentally determined unit cell dimensions of β glycine.

Figure 602
Diffraction pattern of the hk0 plane of β glycine.

Figure 603
Diffraction pattern of the h01 plane of β glycine.
### Lattice Distance from Projection

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<th>(d_{h00})</th>
<th>(d_{0k0})</th>
<th>(d_{001})</th>
</tr>
</thead>
<tbody>
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<td>(hk0)</td>
<td>4.616A (\pm) 0.06A</td>
<td>6.20A (\pm) 0.06A</td>
<td>-</td>
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<tr>
<td>(h01)</td>
<td>4.60 (\pm) 0.6</td>
<td>-</td>
<td>4.90A (\pm) 0.06A</td>
</tr>
</tbody>
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### Unit Cell Dimensions

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<th>Known Value</th>
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</thead>
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<td>(a)</td>
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<td>5.077A</td>
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<tr>
<td>(b)</td>
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<td>6.267</td>
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<tr>
<td>(c)</td>
<td>5.33 (\pm) 0.1</td>
<td>5.397</td>
</tr>
<tr>
<td>Angle between (a^<em>) and (c^</em>)</td>
<td>67.0° (\pm) 0.2°</td>
<td>66.8°</td>
</tr>
</tbody>
</table>

Table 601.
different crystals, the difficulty is the variation of thickness with different crystals.

Besides internal calibration, one can calibrate the general response behavior of the photographic emulsion to electron beams. Since it is very difficult to time the extremely short exposure-times that are needed at the low intensity end of the calibration curve, an electronic device has been constructed which sweeps the electron beam across the photographic plate with an exponential acceleration time. It is basically a RC circuit and its behavior is shown in Figure 601. The output is governed by the equation

\[
V = V_o \left[1 - \exp\left(-\frac{t}{RC}\right)\right]
\]

602

\[
i = \frac{V}{R'}
\]

602a

where

- \(V\) = voltage across the capacitor
- \(V_o\) = maximum voltage
- \(i\) = current
- \(t\) = time
- \(R\) = resistance
- \(R'\) = load resistance at the output
- \(C\) = capacitance

The equation 602a is a valid approximation within the range of time constants used. The current \(i\), which the circuit sends out in the exponential manner as described by equation 602, is used to drive the scanning of the electron beam. The more current that is supplied to the deflection
coils, the greater will be the deflection of the beam. Therefore, the electron beam is deflected across the photo-
plate by the current $i$. The amount of deflection is pro-
portional to $i$. Hence Eq. 602a can be written as

$$X = \ln(1 - \exp(\frac{-t}{RC}))$$  

where  

$$X' = \frac{X'}{X_0}$$

$$X = \frac{X'}{X_0}$$

$X'$ = the distance deflected corresponding to the deflection current $i$.  

$X_0$ = the maximum deflection which depends on the overall gain of the amplifier.

Eq. 603 can be re-arranged to give the amount of time that the electron beam spends within a small increment of distance

$$\Delta t = RC \ln((1-x_1)/(1-x_2))$$  

where  

$$\Delta t = RC \ln((1-x_1)/(1-x_2))$$  

Figure 602 is a plot of $\Delta t$ against $X$; it shows how much time the beam spend with the position along the path. The RC value can be changed to cover a wide range of times.

A tracing of the deflected electron path is then done on the microdensitometer. The microdensitometer is a "double beam" instrument in which two beams of light are used, one passing through the film to be measured and one through a reference wedge, which is a continuous density wedge. The
instrument measures optical density and the reading of the tracings are linear, i.e., if one peak is twice as high as another, then the D of the former peak is twice that of the latter.

Since the tracing is along the path of the electron beam deflection, there is a one to one correspondence between the optical density measured at each position and the time interval $\Delta t$ which corresponds to the position $(X)$. The plot of the optical density or $D$, the photographic blackening, against it gives the desired calibration curve.

In general, $D$ is defined as

$$D = f(It)$$  \hspace{1cm} (605a)

where $I$ is the intensity of the beam and $t$ is the time of exposure. In this experiment, $D$ is related to the optical density by a constant of proportionality

$$D = k[OD]$$  \hspace{1cm} (605b)

where $k$ is a constant dependent on the range of the optical wedge used in the microdensitometer and $OD$ denotes optical density.

In the present experiment, only the lower ranges of $D$ is plotted in the above mentioned manner. For longer exposure times a direct, timed-spot exposure is used. The total calibration curve is shown in Figure 606. Assuming that the electron current density is constant in time, the exposure is then proportional to $t$, so that

$$I = I_e \cdot \Delta t$$  \hspace{1cm} (606)
Figure 604
Oscilloscope trace showing a typical curve of the current $i$ used to deflect the electron beam. The upper and lower graphs are of different RC values.

Figure 605
Typical curve showing the time duration $\Delta t$ which the beam spends at each interval $X+\Delta X$ along the path of the sweep.

Figure 606
Calibration curve of the photographic blackening and the time of exposure. It is used to correct for nonlinearity effects in the data collection and conversion systems.
Figure 606.
where I is the integrated intensity of the diffraction spot, and \( I_e \) is the electron density.

The shape of the curve in Figure 604 confirms the result of Vainshtein [39] in that in the low intensity region there is a "toe" which differs from the exponential behavior of the higher intensity regions. At very high intensity regions when saturation effects occur, the curve would plateau out. However, Valentine [41] reports that the initial slope of the response of photographic emulsion to electrons in the energy range 40 to 80 kv is linear. Our result appears superficially different from this. The explanation for this difference probably lies in the range of optical densities covered. Or as Valentine suggests, a deviation from non-linearity could be due to the fact that because of small penetration (about 15\( \mu \) for 60KV electrons) only the surface layer is exposed and thus the saturation density is low.

1. Correction for Non-linearity

Most of the intensity data fall on the exponential region, some however, fall on the "toe" region. The curve was therefore assumed to consist of two exponential regions with two different slopes. The slopes of the "normal" exponential region and that of the "toe" region are taken separately with the joining point at \( D = 2.0 \). The correction is applied according to the formula

\[
I = C \exp(kD)
\]
where \( k \) is the slope of either the "normal" or of the "toe" region depending on whether \( D \) is larger or smaller than two. \( C \) is a scale factor determined by the boundary position of \( D = 2 \) where the two slope regions meet. Eq. 607 is applied to each diffraction spot, with \( C = 1 \) if \( D = 2 \).

2. Correction for Angular Distribution

Assuming that the specimen is a mosaic single crystal [42] with a uniform angular distribution, the diffraction spot intensity is divided by a factor, which in the case of uniform angular distribution has the form

\[
L = \frac{d_{hkl}}{\alpha} \tag{608}
\]

where \( d_{hkl} \) is the distance between crystal planes defined by the Miller indices \( hkl \), and \( \alpha \) is an angular measure defining the width of the angular spread of the mosaic crystal. Since \( \alpha \) is a constant, it can be disregarded in the correction of relative intensities.

Hence, the intensity of the diffraction spot \( hkl \) is given by

\[
I_{hkl} = \frac{C}{d_{hkl}} \exp(kD) \tag{609}
\]

where \( C \) is some constant which normalizes the two different slope regions and \( k \) can assume either one of the two values of the "normal" or "toe" region. The Bragg spacings are given by

\[
d_{hkl} = \left( k^2 a^2 + k^2 b^2 + l^2 c^2 + 2hla*\cos\beta^* \right)^{-\frac{1}{2}} \tag{610}
\]
where $a^*$, $b^*$, and $c^*$ are the reciprocal space unit cell dimensions and $\beta^*$ is the angle between $a^*b^*$ and the $b^*c^*$ planes of the reciprocal lattice.

It must be noticed that $I_{hkl}$ is only the relative intensity and not the absolute intensity. The structural amplitude is then given by

$$\phi_{hkl} \sim \sqrt{I_{hkl}}$$

or

$$\phi_{hkl} = \frac{S \Omega}{\lambda} \sqrt{I_{hkl}}$$

where $S$ is a scale factor which brings the $\sqrt{I}$ into absolute units consistent with $\phi$. $\Omega$ is the unit cell volume in real space and $\lambda = 0.0418$ Å which is the wave length of the electron.

The square root of the diffraction intensities which are the experimental structure amplitudes are listed in Appendix B together with the calculated structure amplitudes of the reference structure. The values of the $I_{hkl}$ are used as input for the program INCOR.
PART III:

RESULTS
VII. STRUCTURAL DETERMINATION PROGRAMS

It is beyond the scope of this dissertation to give a full or even a reasonable treatment of structural determination programs. All of the programs used in connection with this work are library programs developed and modified by many different researchers. They are all developed for x-ray diffraction work; however, they can easily be adapted to be used for electron diffraction. This chapter will only attempt to describe briefly the logic of the programs and the manner in which they are used in connection with our experiments.

There are three main programs used, all of which were kindly supplied by Dr. Zalkin of the Lawrence Radiation Laboratory at Berkeley. The programs are named INCOR, XRAY (LSLONG) and FORDAP respectively. The way in which they are used is outlined in Figure 701.

INCOR

The program INCOR is normally used to process raw x-ray data and repack them for input uses for either XRAY or FORDAP. It processes both goniostat or weissenberg data, which are not applicable in electron diffraction. Program INCOR also subtracts background and omits forbidden reflection if instructed besides calculating the structural amplitude $\phi_{hkl}$ from input raw intensities.
Figure 701
Diagram showing how the different programs are linked together. See text (Chapter VII) for an explanation of the various programs.
Raw data

INCOR

Tape 5

LSLONG

Tape 5

FORDAP

Tape 7

Least square fitted parameters

Fourier synthesis plots

Figure 701.
For the present work, this program is mainly used to calculate \( F_{\text{obs}} \), which is the symbol designating the observed structural amplitude \( \phi_{\text{hkl}} \), and to exclude unwanted intensities in the systematic absences. Its output is on magnetic tape called Tape 5.

**LSLONG**

Its original name is LS. Tape 5 from INCOR is the input to LSLONG. It basically is a Least Square Program which calculates the phase and the scattering amplitude \( \phi_{\text{cal}} \) from the input atom coordinates and atom scattering tables according to Eq. 218 and 220. Then it compares \( \phi_{\text{cal}} \) with \( \phi_{\text{obs}} \) which is inputted from INCOR for each hkl. It calculates a new scale factor, changes the atom coordinates and the temperature factor by a small increment and calculates a new set of \( \phi_{\text{cal}} \). For each new attempt, it also calculates a R value which is a figure of merit for least square fitting known as the reliability factor (the reliability factor is further discussed in Ch. IX). The program does this reiteratively for as many cycles as instructed. The atom coordinates and the temperature factors of the last cycle define the best fitted structure according to the input data at that particular stage of refinement.

The program converges if the last shifts in the refining parameters are small and the R value no longer decreases appreciably.
The output is written on magnetic tape called tape 7.

FORDAP

FORDAP is essentially a Fourier syntheses program coupled with a plotting routine. As input, it can take either Tape 5 from INCOR or Tape 7 from LSLONG, or from similar data punched on IBM cards if instructed to do so. If the input is Tape 5, then this program sums $\phi^2$ in the Fourier synthesis, thereby calculating the Patterson Function. In the input is Tape 7, the program calculates the distribution of scattering potential according to Equation 213. In addition it can calculate the difference Fourier, or it can do the Fourier summation of the observed structure amplitudes with the phases of the calculated structure. FORDAP also plots, using the CAL-COMP plotter, either sections of projections of the Fourier synthesis, with either $\phi$ or $\phi^2$ depending on instruction from the programmer. By putting in three dimensional data it can even calculate and plot sections through an oblique section.

For a more complete discussion of the general topic of computing methods used in crystallographic work, refer to standard texts such as Computing Methods in Crystallography, edited by J. S. Rollett.
VIII. THE PATTERSON PLOT

The raw data, after the background and the systematic corrections were made as mentioned in Chapter V, is then fed into the program INCOR. The output of INCOR, after rearranging the data and putting them on magnetic tape, serves as the input to the program FORDAP which plots the Patterson Function. The plots for the h0l, hk0, and 0h1 are shown in Figures 801a, 801b, and 801c respectively. The peak positions calculated from the FORDAP plots are listed in Tables 801a, b, and c respectively.

The Patterson Plot, interpreted kinematically, is the auto-correlation function of the scattering potential Eq. 225. If the structure is known, a "point Patterson" can be calculated by plotting the differences of the known coordinates of the atoms (inter-atomic vectors). The plot of these inter-atomic vectors should be similar to the experimental Patterson Plot. In this case, the β glycine coordinates as determined by Iitaka by x-ray diffraction are used as a standard to plot a "point Patterson" map. The x,y,z, projections are shown in Figures 802a, 802b, and 802c respectively.

A visual comparison of the two plots shows that essentially they are similar with some systematic omissions.
Figure 801a

h01 projection of the Patterson Function of β glycine. The peaks are listed in Table 801a.

Table 801a

Peak positions of the Patterson Function, h01 projection. This is a printout from program INCOR. RHO is the value of the peak heights. A projection of these peaks is plotted in Figure 801a.

Figure 802a

"Point Patterson" map. h01 projection. This is calculated by plotting points of the interatomic vectors from the known atom positions of the molecules within one unit cell.
### Table 801-A

#### 1st of Peak Positions and Their Heigths, Rhodaruf Run x 999, / Rhod Max.

Syntactically duplicated peaks are indicated in parentheses. (1949) indicates a shoulder and not a true peak.

<table>
<thead>
<tr>
<th>No.</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>True Run</th>
<th>Interpolation Peaks</th>
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- **Fvel of the last section = 1.000**
- **N of complete sections = 11**
- **N of plots = 1**

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Translation (natural text):

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<th>Y</th>
<th>Z</th>
<th>True Run</th>
<th>Interpolation Peaks</th>
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</thead>
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<tr>
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</tr>
<tr>
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<td>0.000</td>
<td>999 (195.40)</td>
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<td>999 (195.40)</td>
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<td>0.000</td>
<td>999 (195.40)</td>
<td></td>
</tr>
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</table>
Figure 80lb

hk0 projection of the Patterson Function

Table 80lb

Peak position of the Patterson Function, hk0 plane. This is a printout from program INCOR. RHO is the value of the peak heights. A projection of these peaks is plotted in Figure 80lb.

Figure 802b

hk0 projection of the "Point Patterson." This is calculated by plotting points of the interatomic vectors from the known atom positions of the molecules within one unit cell.
Table 801.2

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</table>

SYNTACTICALLY Duplicated Peaks ARE Indicated IN PAIREDNESS. (1991) TO indicate a SHOULDER AND NOT A TRUE PEAK.
Figure 801c

0hl projection of the Patterson Function. The peaks are listed in Table 801c.

Table 801c
Peak positions of the Patterson Function. 0kl projection. This is a printout from program INCOR. RHO is the value of the peak heights. A projection of these peaks is plotted in Figure 801c.

Figure 802c

0kl projection of the "Point Patterson." This is calculated by plotting the points of the interatomic vectors from the known atom positions of the molecules within one unit cell.
**List of Peak Positions and Their Heights**: For Table 801-C

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<thead>
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<th>Y</th>
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<td>2.500</td>
<td>2.500</td>
<td>420</td>
<td></td>
</tr>
</tbody>
</table>

**Legend**
- "X" gives the X coordinate of the peak.
- "Y" gives the Y coordinate of the peak.
- "Z" gives the Z coordinate of the peak.
- "Peak" gives the height of the peak.
- "Interpolated Peaks" indicates the interpolated values for X, Y, Z, and Peak.

**Table 801-C**

- **Number of Peaks**: 6
- **Level of Last Section**: 1.600
- **Interpolated Sections**: 11
- **Level of the Last Section**: 1.600
Two conclusions are possible:

(1) The diffraction patterns have no significant dynamical effect and hence can be interpreted on purely kinematical grounds. Their Patterson plots are similar to what they should be theoretically; the omissions means that certain interatomic distances are not resolved well.

(2) The diffraction patterns have significant dynamical effect, but the Patterson function is not sensitive enough to show the difference. This is plausible because the Patterson is a convolution of two functions. Even if these functions are modified, as in the case of dynamical effect, so that the $\psi$ is modified by a factor $K$, according to the two-beam approximation discussed below, and would appear differently from that of the Kinematic $\psi$, the convolution function would not change appreciably.

From the consideration of the two-beam dynamical approximation [43] in the case of a medium thickness block, the integrated intensity can be expressed as

\[ I_{hkl} = C \lambda^2 |\phi_{hkl}|^2 \cdot K(\xi, \phi_{hkl}) \cdot \Gamma \]

where $C$ is some constant, $\lambda$ is the wavelength, $\phi_{hkl}$ is the structural amplitude. $\Gamma$ is a factor which takes into account the mosaic nature of the sample and is proportional to $d_{hkl}$, the plane distance.

\[ K(\xi, \phi_{hkl}) = \exp \left( -\frac{1}{3} \xi^2 \frac{\phi_{hkl}}{\Omega} \right) \]

and $\Omega = \text{volume}$, $\xi = \text{the thickness of the crystal}$. Eq. 801
can be rewritten as

\[ I_{\text{hkl}} \sim \phi_{\text{hkl}}^2 \cdot K \]  

The Patterson function is defined as

\[ P = F^{-1}(I) \]

where \( I \) is omitted for convenience and \( F^{-1} \) denotes the operation of a Fourier summation and the subscripts are dropped for convenience. Therefore

\[
\begin{align*}
P & \sim F^{-1}(\phi^2 \cdot K) \\
& \sim F^{-1}(\phi^2) \cdot F^{-1}(K) \\
& \sim P_k \cdot F^{-1}(K)
\end{align*}
\]

where \( P_k \) denotes the Patterson in the kinematic case and \( \cdot \) denotes the convolution operator. This shows that the Patterson is further convoluted with the transform of the modification factor.

Alternately, if \( \phi' \) is the "true" kinematic structure amplitude, and \( \phi \) the dynamical or apparent structure amplitude and \( K \) the two-beam correction factor as defined in Eq. 802, then \( \phi' \) can be expressed as

\[ \phi' = \phi \cdot K^{-1} \]

The Patterson in this case is then

\[ P' = F(|\phi'|^2) \]

The correction factor \( K \) is plotted in Figure 803.

As shown by Eq. 806, \( K \) modifies \( \phi \); but according to
Figure 803, its effect is only considerable if $\Phi$ is large. It is readily seen that $K^{-1}$ only increased the amplitude of $\Phi$ with no other effect. Eq. 807 indicates that the Patterson function is now calculated with each intensity increased by a factor $K^{-1}$. In the case of the modification being small, i.e., $K = 1$, it is seen that according to the two beams approximation, the Patterson map will not significantly differ from the kinematical Patterson and therefore does not show any definite effects in the locations of peaks because of dynamical scattering. For a multiple beam dynamical approximation such as that published by Schiff [44] the modified Patterson is more difficult to interpret. According to Schiff, the structural amplitude can be expressed as

$$\Phi \sim iF(1-e^{-i\psi})$$

where $\psi$ is the electric potential within the unit cell.

The Patterson is then

$$P = F^{-1}[\Phi \cdot \Phi^*] \sim F^{-1}(I)$$

$$= F^{-1}[F(1-e^{-i\psi(x,y)}) F^{-1}(1-e^{i\psi(x,y)})]$$

$$= (1-e^{-i\psi(x,y)})(1-e^{i\psi(-x,-y)})$$

It is the convolution of a complex function of the electric potential instead of the autocorrelation of the electric potential. In the case that the dynamical scattering is not very strong, i.e., if $\psi$ is small, the complex function goes over to the kinetic case readily. For small $\psi$ if $\psi \ll 1$, then
Theoretical values of $K$ (after Vainshtein, ref. [3]). This is the factor which corrects for dynamical effect according to the two-beam approximation. When $\xi \cdot \lambda \cdot |\phi| < 2K$ can be approximated as $\exp(-\gamma_3 \frac{\xi}{\xi - 2} \lambda^2 |\phi|^2)$.
Figure 803.
\[ i(1-e^{-i\psi}) \rightarrow i(1-l+i\psi) \rightarrow \psi \]

Therefore, in the case of not very strong scattering, the Schiff formula approaches the kinematic formulation and we would not expect the Patterson to differ appreciably from the kinematic case also.

According to the theory of Cowley and Moodie [45] for the case of a one dimensional centrosymmetric crystal with Gaussian atoms

\[ \psi(x, y) = c \exp(-x^2) \]

the Patterson function can be expressed as (see Eq. 229)

\[ P = \exp(\text{ice}^{-x^2}) \exp(\text{ice}^{-x^2}) \]

where \( c \) is some number which represents variations in the atomic number of the atoms or the crystal thickness. For the cases of light atoms \( c \) is less than 2. The way in which the peak heights and the total areas of the peaks of Eq. 812 vary with \( c \) is given by Figure 804. It is seen that for light atoms (with \( c \) less than 2) the dynamical Patterson is not appreciably different from the kinematical Patterson.

The present experiment uses more accurate atomic scattering factors and three dimensional data. The crystal of \( \beta \) glycine is centrosymmetric. Therefore the analysis of Cowley and Moodie has relevance even though their calculation is one dimensional and is based on an assumed Gaussian scattering potential. The experimental observation reported here seem to confirm their result.
Figure 804

The variation with $c$ of the peak height and peak area for Patterson Peaks [9]. $c$ is some number which represents either the atomic number or the atoms or the crystal thickness. This graph shows the difference between the Patterson function calculated with the kinematic theory and that which was calculated by the dynamic approximation of Cowley and Moodie.
Figure 804.
IX. STRUCTURE OF β GLYCINE

The raw intensity data, after correcting for the non-linearity previous to the digitizing process (Chapter VI), and after being arranged by the program INCOR, are used as input to the Least Square Structural Factor Program XRAY (LSLONG). The logic and mechanics of the programs are discussed briefly in Chapter VII. For a more detailed discussion of structural programs of this type, we refer to Stout and Jensen [32] and other standard x-ray crystallography textbooks.

Procedure

1. Use the atomic scattering factors for electrons f_{el} to calculate the structure amplitudes \( \phi_{hkl} \) of the "trial" structure. The "trial" structure in this case is our reference structure determined by Iitaka using x-ray methods. We have put in as input coordinates the atom coordinates as published by Iitaka in 1959 [24]. The \( \phi_{hkl} \) thus calculated serves as the basis for determining to what extent the diffraction data show dynamical effects. The result of this experiment is discussed in Chapter X.

2. Using the Iterative Least Square Procedure, to see how well the diffraction data compare with the trial structure. This program calculates the atom coordinates of a new
structure whose electron scattering amplitudes $\psi_{hkl}$ are the most consistent with the diffraction data as determined by the Least Square Method. The program also calculates a new temperature factor for each atom; in this case we have only attempted the isotropic case. The initial temperature factor should be determined by a Wilson plot, but for simplicity we have put in as input the temperature factor as given by Iitaka.

(3) By putting a small variation of the atomic coordinates in such a trial structure from the standard reference structure we can see whether the diffraction data converges to the same minimum or whether they converge to a different minimum. If they converge to a different minimum, this would indicate that the set of experimental data is not good enough to give a unique structure and hence, we would have less confidence in this experimental procedure.

(A) Reliability Factor: After a five cycle refinement, the program converges with a reliability factor $R = 0.48$, the $R$ value for modern x-ray crystallography technique is for most cases less than 10% and for careful work can be refined to as low as 1-2%. A. J. C. Wilson showed that the theoretical value for $R$ which would be obtained by using the proper kind and number of atoms placed randomly is $R = 0.59$ for acentric space groups and for centric space groups $R = 0.83$ [45][46]. Vainshtein [47] has pointed out.
that for electron diffraction the reliability factor should be about 1.5 times higher in the absence of termination of series for a three dimensional series and 2 times higher for projections than x-ray diffraction.

However, in any experiment, a R value more than 20-30% is indicative of the fact that low levels of confidence should be given to the structure. The reliability is defined as

\[ R = \frac{\sum |\phi_o| - |\phi_c|}{\sum |\phi_o|} \]

where \( \phi_o \) and \( \phi_c \) denote the observed and calculated structural amplitudes respectively. This parameter shows the degree of agreement between the observed and calculated data. Even though the R value does not assure the correctness or incorrectness of a structure, at least a large R value shows poor agreement with the data.

(B) Bond Lengths and Bond Angles: The atom positions given by the program LSLONG are calculated with the coordinates of the atom N being instructed to be fixed in the program so that an origin of the coordinate system of the unit cell can be defined. The atom coordinates from the calculated structure are tabulated in Table 901, and that of the reference structure in Table 901b. The bond lengths and bond angles calculated respectively from these coordinates are listed in Table 902a, b. Table 902b also lists the differences in bond lengths and bond angles between the values
Figure 901
Molecule of β glycine
Figure 901.
Table 901a
Atom coordinates of β glycine in fractional units of the unit cell dimensions as given by Iitaka.

Table 901b
Atom coordinates in fractional coordinates as calculated by the present experiment. Hydrogen atoms not included in the structure factor program.
### Table 901a

<table>
<thead>
<tr>
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<th>x</th>
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<th>z</th>
</tr>
</thead>
<tbody>
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<td>-0.0440</td>
<td>-0.2619</td>
</tr>
<tr>
<td>O1</td>
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<tr>
<td>Cl</td>
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<td>0.0633</td>
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<tr>
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### Table 901b

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<td>-0.0440</td>
<td>-0.2619</td>
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<td>O2</td>
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<td>0.1248</td>
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<td>0.0756</td>
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<tr>
<td>C2</td>
<td>0.1095</td>
<td>0.1230</td>
<td>-0.2396</td>
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</table>
Table 902a

Bond lengths and bond angles, calculated by using the atom coordinates of Table 901a.

Table 902b

Bond lengths and bond angles, calculated by the atom coordinates of Table 901b. The differences are the differences calculated by subtracting this value from the standard value which is given in Table 902a.
<table>
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<tr>
<th>BOND LENGTHS</th>
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<th>difference</th>
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<tr>
<td>N-C2</td>
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<tr>
<td>C2-C1</td>
<td>1.5207</td>
<td></td>
</tr>
<tr>
<td>Cl-01</td>
<td>1.2279</td>
<td></td>
</tr>
<tr>
<td>Cl-02</td>
<td>1.2464</td>
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<table>
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<th>BOND ANGLES</th>
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<th>difference</th>
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<tr>
<td>C2-C1-02</td>
<td>115.9320°</td>
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<tr>
<td>Cl-C2-N</td>
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</tr>
<tr>
<td>C2-Cl-01</td>
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<td>Cl-01-02</td>
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Table 902a. Bond lengths and bond angles.
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<td>Cl-O1</td>
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<td>Cl-O2</td>
<td>1.3712</td>
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<th>BOND ANGLES</th>
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<th>difference</th>
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<tr>
<td>C2-Cl-O2</td>
<td>105.7590°</td>
<td>10.1734°</td>
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<tr>
<td>Cl-C2-N</td>
<td>93.0441</td>
<td>17.7559</td>
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<tr>
<td>C2-Cl-O1</td>
<td>105.2870</td>
<td>12.4925</td>
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<tr>
<td>O1-Cl-O2</td>
<td>120.0000</td>
<td>6.1792</td>
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</table>

Table 902b. Bond lengths and bond angles.
given by Iitaka and that which is given by the present experiment. The atoms of $\beta$-glycine are labeled in Figure 901. It can be seen readily that the changes in bond lengths are relatively small compared with the changes in bond angles. The largest changes in bond length occurs with the $C_1-C_2$ bond which is 0.27 $\AA$ or about 20%. The largest change in bond angle is the angle at atom $C_2$. The change is 17° or almost 17%. Assuming the correctness of the reference structure, this degree of deviation is very significant.

(C) The Question of Temperature Factor: In the early stages of structure determination, we encountered negative temperature factors in the structural program refinement. Taking the thermal motion of the atoms into account, the structure amplitude can be expressed as

$$\phi_{hkl} = \sum_i f_{el_i,T} \exp(2\pi i \vec{r}_i \cdot \vec{H})$$

where $\vec{r}_i$ is the position vector of atom $i$, $\vec{H}$ is the vector of the reciprocal lattice and $f_{el_i,T}$ is the atomic scattering factor of the $i$th atom multiplied by the thermal function, i.e.,

$$f_{el_i,T} = f_{el_i} f_T$$

$$f_T = \exp(-B(\sin\theta)^2/\lambda^2)$$

where $B$ is defined as the temperature factor. Unreal or negative temperature factors can be due to the following situations.
(1) Uncorrected non-linear effects of the data processing procedure.

In Chapter VI, it has been shown that the response of the photographic plate is non-linear. For the most part, a correction of the form \( I = e^{KD} \), where \( D \) is the blackening and \( K \) is some constant, is adequate. Suppose that the slope \( K \) is corrected, and \( K' \) is the true slope, then Eq. 904 can be written as

\[
I = e^{KD} = e^{(K'-\Delta K)D} = e^{K'D}e^{-\Delta KD}
\]

recalling that

\[
\phi \cdot U^I = \sqrt{e^{KD}e^{-\Delta KD}} = \sqrt{I} \cdot e^{-1/2\Delta KD}
\]

\[
\therefore \sqrt{I} = \phi \cdot e^{1/2\Delta KD}
\]

Equation 906 shows that in this case the effect is the introduction of a factor of the form \( e^{1/2\Delta KD} \) into Eq. 902. This factor also varies although in some complicated manner, as \((\sin^\theta/\lambda)^2\) and it also counteracts the temperature factor.

(2) The atomic scattering factor \( f_{el} \) as a function of \((\sin^\theta/\lambda)^2\) falls off too slowly. This needs a re-calculation of the \( f_{el} \) tables.

The \( f_{el} \) as a function of angle is shown in Figure 902. It can be seen from Figure 902 that \( f_{el} \) approximates an \( e^{-K(\sin^\theta/\lambda)^2} \) curve. If it is falling off less sharply than it should, the effect is as though \( f_{el} \) is modified by some factor of the form \( e^{+K'(\sin^\theta/\lambda)^2} \) with some constant \( K' \). This
factor has the same functional form as \( f_T \) and counteracts to it.

(3) Dynamical effects

For a single crystal of "medium" thickness, the integrated intensity according to the two beam dynamical approximation can be expressed as

\[
I = c\lambda^2 |\Omega|^2 \cdot t \cdot K
\]

where \( c \) is some constant, \( t \) is the thickness of the crystal, and

\[
K = \exp\left\{-\frac{1}{3}(t)^2 \cdot \lambda^2 |\Omega|^2 \right\}
\]

with \( \Omega \) denoting the unit cell volume.

If \( I' \) is the correct intensity \( I' \sim \phi^2 \) and

\[
\phi \sim \sqrt{I'} = \sqrt{I} \cdot \frac{1}{K}
\]

It is obvious from Eq. 908 that the dynamical correction factor \( 1/K \) introduces into Eq. 902 a factor of the form \( e^{+k\phi^2} \) which also is dependent on \( (\frac{\sin \theta}{\lambda})^2 \) and counteracts the temperature function \( f_T \).

From the experimental results Case (2) clearly does not apply. If Case (2) were true, then the temperature factor of the same kind of atom, for example carbon, will be negative. The results of three types of experiments directed to this question are tabulated in Tables 904, 905, and 906. The experimental results as listed: Tables 904 and 905 are calculated from the same raw intensity data but their
intensities are corrected for non-linearity by different values of slope $K$.

From Tables 904 and 905, it is shown that the temperature factor of Cl and C2 are of different signs. Therefore, case (2) is ruled out.

Case (1) seems probable because, as shown in Table 906, after correction of non-linearity effect by assuming a two slope correction curve (Chapter VI) all the temperature factors become positive. While the correction of non-linearity as described in Chapter VI yields positive temperature factors, it does not improve the $R$ value. This means that the reliability for the calculated structure is lower than before.

The increase in $R$ value with positive temperature factors may mean that the correction method for the non-linearity of the calibration curve is not adequate and introduces systematic errors which cause the increase in $R$. This explanation seems acceptable because, as was pointed out in Chapter VI, a point by point correction according to the calibration curve should give much more reliable results than the two slope systematic correction method we have used.

Another explanation for the negative temperature factor is Case (3). The evaluation of the extent to which the diffraction data are dynamical will be discussed in Chapter X.

A combination of the effects of Case (1) and Case (3) is possible.
Figure 902

The atomic scattering factor $f_{el}$ as a function of $\sin \theta / \lambda$ (after Vainshtein, ref. [3]).
Output of the Least Square program XRAY (LSLONG). Listed are: The reliability factor $R$, the scale factor, the new atom coordinates $x$, $y$, $z$, the new temperature factor $B$, and the atom types. This is the result after 5 cycles of refinement.

Table 904

Calculated with a one slope non-linearity correction for the raw intensities.

Table 905

Calculated with a one slope non-linearity correction for the raw intensities. Slope different from Figure 904.

Table 906

Calculated with a two-slope non-linearity correction for the raw intensity data.
Table 904

\[ R \text{ (INCLUDING ZERO WEIGHT DATA)} = .43732 \text{ FOR 235 DATA.} \]
\[ \text{NEW SCALE FACTOR FOR FOBS} = .18774 \]

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Table 905

\[ R \text{ (INCLUDING ZERO WEIGHT DATA)} = .35765 \text{ FOR 199 DATA.} \]
\[ \text{NEW SCALE FACTOR FOR FOBS} = .48993 \]

<table>
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Table 906

\[ R \text{ (INCLUDING ZERO WEIGHT DATA)} = .48925 \text{ FOR 235 DATA.} \]
\[ \text{NEW SCALE FACTOR FOR FOBS} = .18657 \]

<table>
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<td>.123050</td>
<td>-.239613</td>
<td>.703</td>
</tr>
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</table>
(D) **Convergence and the Number of Minima:** To get some idea about the convergence behavior of the data, as determined by the least square program, three experiments are performed under the following conditions:

1. A small random variation of magnitude 0.02 fractional units is introduced into the input coordinates of the trial structure,

2. All other parameters are kept unchanged.

The results of such experiments are tabulated in Tables 907 and 908. It is clearly seen that they all converge to different minima with high R values.

These experiments add another testimony to the unreliability of the result.

It is not unusual that least square programs do sometimes converge to different minima, for variation in input coordinates of ± 0.02 fractional units, or about 0.1 Å. This kind of variation is not too unreasonable if the atom positions are not accurately known beforehand. If there were enough data to completely overdetermine the parameters, the programs should converge to the same minimum. In the present investigation, the three dimensional data together have about 235 different spots. This is equivalent to having about half that amount of unique data points because of symmetry. When this number is compared with the situation in x-ray diffraction, where data usually run into thousands, our technique at this stage of development seems crude indeed.
Output of the Least Square Program XRAY (LSLONG). Listed are: The reliability factor \( R \), the scale factor, the new atom coordinates \( x, y, z \), the new temperature factor \( B \), and the atom types. This is the result after 5 cycles of refinement.

**Table 907a**

Result of a slight variation in input trial structure. Experiment I.

**Table 907b**

Result of a slight variation in input trial structure. Note the unphysical 7 coordinate. Experiment II.

**Table 907c**

Result of a slight variation in input trial structure. Note the unphysical 7 coordinates. Experiment III.
**FIG. 907a**

\[
\begin{align*}
R \text{ (INCLUDING ZERO WEIGHT DATA)} &= .53306 \text{ FOR 235 DATA.} \\
\text{NEW SCALE FACTOR FOR FOBS} &= .16976 \\
\end{align*}
\]

<table>
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<th>NEW Z</th>
<th>NEW B11 OR B</th>
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<td>5</td>
<td>.131034</td>
<td>.131650</td>
<td>-.391769</td>
<td>4.359</td>
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</table>

**FIG. 907b**

\[
\begin{align*}
R \text{ (INCLUDING ZERO WEIGHT DATA)} &= .49739 \text{ FOR 235 DATA.} \\
\text{NEW SCALE FACTOR FOR FOBS} &= .19577 \\
\end{align*}
\]

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<td>.027822</td>
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<td>.103518</td>
<td>.117382-33.275909</td>
<td>-.534</td>
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</tr>
</tbody>
</table>

**FIG. 907c**

\[
\begin{align*}
R \text{ (INCLUDING ZERO WEIGHT DATA)} &= .48432 \text{ FOR 235 DATA.} \\
\text{NEW SCALE FACTOR FOR FOBS} &= .18574 \\
\end{align*}
\]

<table>
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<th>NEW Z</th>
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<td>5</td>
<td>.103031</td>
<td>.127044-38.264245</td>
<td>1.419</td>
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</table>
Table 908a
Bond lengths and bond angles. Result of variation of input Experiment I. Calculated with the atom coordinates given in Table 907a. The difference is that between this result and the known values.

Table 908b
Bond lengths and bond angles. Result of variation of input Experiment II. Calculated with the atom coordinates given in Table 907b. The difference is that between this result and the known values. Unphysical values in the bond lengths are due to flaws in the least square program which allow the atom coordinates to exceed the unit cell dimension.

Table 908c
Bond lengths and bond angles. Result of variation of input Experiment III. Calculated with the atom coordinates given in Table 907c. The difference is that which is between this result and known values. Unphysical values in bond lengths are due to flaws in the least square program which allow the atom coordinates to exceed the unit cell dimension.
### Table 908a. Bond lengths and bond angles.

<table>
<thead>
<tr>
<th>BOND LENGTHS</th>
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<th>difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-C2</td>
<td>1.5124 Å</td>
<td>-0.0287 Å</td>
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<tr>
<td>C2-C1</td>
<td>1.5786</td>
<td>-0.1579</td>
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<tr>
<td>Cl-O1</td>
<td>1.2686</td>
<td>-0.0407</td>
</tr>
<tr>
<td>Cl-O2</td>
<td>1.6095</td>
<td>-0.3631</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>BOND ANGLES</th>
<th>calculated</th>
<th>difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2-C1-O2</td>
<td>116.4200°</td>
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<tr>
<td>Cl-C2-N</td>
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<td>41.127</td>
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<td>C2-C1-O1</td>
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<td>O1-C1-O2</td>
<td>109.4600</td>
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Table 908b. Bond lengths and bond angles.

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<tbody>
<tr>
<td>N-C2</td>
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</tr>
<tr>
<td>C2-C1</td>
<td>*</td>
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<td>Cl-01</td>
<td>1.3378</td>
<td>-0.1099</td>
</tr>
<tr>
<td>Cl-02</td>
<td>1.3395</td>
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</table>

<table>
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<td>Cl-C2-N</td>
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<tr>
<td>C2-C1-01</td>
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<tr>
<td>Cl-01-02</td>
<td>109.8330</td>
<td>16.3457</td>
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* Unphysical values (please see legend)
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<tr>
<th>BOND LENGTHS</th>
<th>calculated</th>
<th>difference</th>
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<tr>
<td>N–C2</td>
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<tr>
<td>C2–Cl</td>
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<td>Cl–O1</td>
<td>1.2616</td>
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<tr>
<td>Cl–O2</td>
<td>1.3828</td>
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<table>
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<td>C2–Cl–O2</td>
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<td>Cl–C2–N</td>
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<tr>
<td>C2–Cl–O1</td>
<td>109.8960°</td>
<td>−7.8828</td>
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<tr>
<td>O1–Cl–O2</td>
<td>120.2668°</td>
<td>−5.9132</td>
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</table>

*Unphysical values (please see legend)*

Table 908c. Bond lengths and bond angles.
The number of unique data points used in Iitaka's work was only about one hundred, however. His data were from the h0l and 0kl projections. This number should be looked upon as the barest minimum that can be used, as present day x-ray crystallography usually works with data points numbering one or two orders of magnitude higher.

(E) Hydrogen Atoms: A Fourier synthesis using the phases and scattering amplitudes of the calculated structure is shown in Figure 902. A ΔF synthesis using the same conditions is shown in Figure 903.

The difference Fourier synthesis is a powerful tool in the refinement of the atom parameters. It is a Fourier synthesis using the difference between the magnitudes of the observed and calculated structure amplitudes |φ₀| - |φ₁| as coefficients. It can be expressed in the form

\[ ΔF = \sum_{hkl} (|φ₀| - |φ₁|) e^{iα} e^{-2π(\hat{H} \cdot \hat{r})} \]

where \( α \) is the phase of \( φ₁ \). If the phases are correct, Eq. 909 provides a direct measure of the errors between the model used and the "true" structure implied by the \( φ₀ \)'s.

The difference map has a peak everywhere that the \( φ₁ \) model fails to provide the electron density implied by the \( |φ₀| \) (the phases of the \( φ₁ \) are used). It shows a hole where it provides too much. As a first approximation, correctly placed atoms will not appear in the synthesis, incorrectly placed ones will be in holes and missing ones will appear as
Figure 902
Fourier synthesis map of the scattering potential of β glycine at a section along the b-axis where \( b = 0.8 \) fractional units.

Figure 903
The ΔF synthesis of β glycine along the b axis. It is a section at \( b = 0.8 \) fractional units. The solid dots are the atom centers of the β glycine molecule minus hydrogens. The open circles denote hydrogen atoms. See text for further discussion.
Output of the Least Square Program XRAY (LSLONG). Listed are: The reliability factor R, the scale factor, the new atom coordinates x,y,z, the new temperature factor B, and the atom types. This is the result after 5 cycles of refinement.

Table 909
Hydrogen positions as given by Iitaka are included in the calculation, but they are not refined.

Table 910
Bond lengths and bond angles. Calculated from Table 909. The difference shows the discrepancy between this result and that of the known values.
FIG. 909

R (INCLUDING ZERO WEIGHT DATA) = .47185 FOR 235 DATA.
NEW SCALE FACTOR FOR FOBs = .19515

<table>
<thead>
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<th>NEW Z</th>
<th>NEW B11 OR B</th>
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<td>.590 N</td>
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<td>.039544</td>
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<td>.591 O1</td>
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<td>.105738</td>
<td>.2939 O2</td>
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<td>4</td>
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<td>.077351</td>
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<td>.109340</td>
<td>.114648</td>
<td>-.213692</td>
<td>-.061 C2</td>
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</table>

ATOMS NOT REFINED.

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<td>-.203000</td>
<td>-.216000</td>
<td>2.000 H3</td>
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<td>10</td>
<td>-.091000</td>
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<td>-.362000</td>
<td>2.000 H5</td>
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### Bond Lengths

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<td>N-C2</td>
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<td>-0.1832 A</td>
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<tr>
<td>C2-Cl</td>
<td>1.6189</td>
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</tr>
<tr>
<td>Cl-01</td>
<td>1.2378</td>
<td>-0.0099</td>
</tr>
<tr>
<td>Cl-O2</td>
<td>1.3093</td>
<td>-0.0629</td>
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### Bond Angles

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</tr>
</thead>
<tbody>
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<td>C2-Cl-O2</td>
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<td>13.1883°</td>
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<tr>
<td>Cl-C2-N</td>
<td>100.3580</td>
<td>10.4422</td>
</tr>
<tr>
<td>C2-Cl-01</td>
<td>109.2890</td>
<td>8.4904</td>
</tr>
<tr>
<td>O1-Cl-O2</td>
<td>124.8320</td>
<td>1.3475</td>
</tr>
</tbody>
</table>

Table 910. Bond lengths and bond angles.
peaks.

It also shows where an anisotropic temperature factor is needed. For further discussion on the role of the $\Delta F$ synthesis in the refinement of structures, the reader is referred to standard texts such as Stout and Jensen [48].

From the difference Fourier map of Figure 903 it is readily seen that

1. Atom O1 has too large an isotropic temperature factor (TF) and anisotropic TF correction is necessary,

2. Atom Cl has too small an isotropic TF and also anisotropic TF correction is needed,

3. Atom O2 needs a larger shift in coordinates, and/or probably has too large a temperature factor,

4. Hydrogen atoms are easily located and occur at peaks labeled with open circles and connected by dotted lines as shown in Figure 903.

These observations indicate that further refinements in the direction of

a. Putting in anisotropic temperature factors for at least atoms O1 and Cl.

b. Including hydrogen atom positions are in order.

But as mentioned above, because of the high reliability factor value, further refinement was not attempted.

The above observations testify to the fact that the scattering cross-sections of hydrogen with electron diffraction is considerably higher than that of x-ray diffraction,
hence detection is much easier.

Except for the fact that a couple of the smaller peaks in the difference map (Figure 903) cannot be accounted for, Figure 903 is an excellent example of the detection of hydrogen atom locations by the difference Fourier synthesis of electron diffraction methods.

Figure 903 further suggests that including the hydrogen atoms into the calculation of structural amplitudes can reduce the error. The result is shown in Table 908, in which a 5 cycle refinement of the least square program with the hydrogen atoms included but not refined is tried.

As shown in Table 909 and 910, this experiment produces a slightly better result, as the calculated bond lengths and bond angles are concerned, but it neither reduces the R factor nor gives a reasonable temperature factor. The conclusions drawn previously in this chapter therefore still hold.

(F) Refinement of Calculated Structure: In view of the preceding results, refinement of the calculated structure seems out of the question. Therefore, we have decided not to further refine by the normal techniques such as using anisotropic temperature factors, doing difference Fourier synthesis and refining the positions of the hydrogen atoms, etc.
X. EXTENT OF DYNAMICAL SCATTERING

The first sign indicating possible effects of dynamical scattering is the presence of forbidden reflections. Since we are working with a structure that has been accurately determined by x-ray diffraction, these forbidden reflections do not present serious problems in space group determination. If the structure is unknown and if there were no accurate x-ray diffraction data to act as a standard reference, the presence of forbidden reflections would pose serious problems in the determination of space groups.

The space group of glycine as by Iitaka is \( P_2_1 \) [24]. This is confirmed by the indexing of our electron diffraction patterns except for the forbidden reflections along the \( k \) axis. The extinction rule is \( k = \) odd, for the \( hko \) reciprocal lattice row of the \( hko \) projection, and none for the \( h0l \) projection. As can be readily seen from Figure 1001, the \( 010, 030, 050, 010, 030, 050 \) reflections do appear with appreciable intensities. In Chapter VI it was pointed out that since we have the x-ray diffraction data as standard, the INCOR program was instructed to ignore these forbidden reflections. However, the appearance of these systematic reflections may be interpreted either as a sign of dynamical effect or as a result of incoherent secondary scattering [9,
Figure 1001
Microdensitometer tracing of the 0h0 reflections. These are values of the diffraction spots before non-linearity connections are made.
27,44]. If the forbidden reflections are due to secondary scattering rather than multiple scattering, it is possible to correct for it if its influence is not very strong [49, 50]. This represents yet another stage of refinement which we shall not attempt to do.

There are methods for correcting the effect of dynamical scattering such as that proposed by Honjo and Kitamura [51] and by Nagakura [52]. Honjo and Kitamura made their dynamical correction based on the two beam approximation of Blackman. They proposed a method of recording data at several wavelengths and extrapolating the observed intensities to zero wavelength. Nagakura proposed another method utilizing Wilson's intensity statistics combined with the theory of primary extinction.

Any effort compensating the effects of dynamic scattering would undoubtedly improve both the resolution and the reliability of structural determination. The correction represents yet another stage in the refinement of the calculated structure.

If a standard reference structure is known and its structure amplitude $\phi$ is calculated, Vainshtein [53,54] suggests a method which allows the comparison of the two beam correction factor $K_{\text{exp}}$ with the theoretical value of that quantity. The $K_{\text{exp}}$, which is arrived at experimentally, also allows a crude calculation of $\bar{t}$, the mean thickness of the crystal. The method is valid for crystals of "medium"
thickness and for the region \( \bar{t} \cdot \lambda |\phi_{hkl}| \leq 2 \).

According to the two beam approximation, the integrated intensity for a "medium" thickness crystal can be expressed as

\[
I_{hkl} = \lambda^2 |\phi_{hkl}|^2 \bar{t} \cdot K(\bar{t}, \phi)
\]

with

\[
K(\bar{t}, \phi) = \exp\left\{ -\frac{1}{3} (\bar{t} \cdot \lambda |\phi_{hkl}|)^2 \right\}
\]

These equations are the same as Eq. 907 and 908 respectively and the symbols are as defined in those two equations.

Equation 1001 can be written as

\[
\frac{k \cdot I_{\text{rel}}}{\phi_{\text{cal}}^2} = c \cdot K_{\text{exp}}
\]

where \( c \) is equal to \( \frac{\Omega^2}{\lambda^2 \bar{t}} \), \( I_{\text{exp}} \) is the experimental relative intensity, and \( k \) is the scale factor which brings \( I_{\text{cal}} \) into the same unit as \( \phi_{\text{cal}}^2 \). Equation 1003 is valid for each hkl reflection in the two beam case. Since Eq. 1002 defines the functional form of \( K \), it follows that \( K_{\text{exp}} \), defined by Eq. 1003, would also have the same functional form if the two beam dynamical approximation for the medium thickness case were applicable. Equation 1002 can be re-written as

\[
\sqrt{-\ln K} = \sqrt{\frac{1}{3} \frac{\bar{t} \cdot \lambda}{\Omega} \phi} = m \phi
\]

Equation 1004 defines \( m \). If we put into Equation 1004 \( K_{\text{exp}} \) instead of \( K \), then the slope of the plot of \( K_{\text{exp}} \) against \( \phi_{\text{cal}} \) is \( m \). With the value of \( m \) thus determined, the average
thickness $\bar{t}$ can readily be obtained from Equation 1004.

The average thickness $\bar{t}$ could also be measured by using the intercept of Equation 1003,

$$k \left[ \frac{\text{rel}}{\exp} \right]_{\phi=0} = \frac{\Omega^2}{\lambda^2 \bar{t}}$$  \hspace{1cm} 1004a

However, in this case $k$ which is the scale factor, would have to be determined independently such as by varying $\lambda$. The plot of $K_{\exp}$ against $\phi_{\text{cal}}$ is shown in Figure 1002. The slope determined from it is $m = 0.2 \pm 0.1$. The average thickness of the crystal is then

$$t = \frac{m \sqrt{3}\Omega}{\lambda}$$  \hspace{1cm} 1005

Substituting into Equation 1005, $\lambda = 0.0418\text{Å}$, $\Omega = 157.835\text{Å}^3$, we obtain $\bar{t} = 1310\text{Å}$, within a factor of two.

This value of the average thickness is of the same order of magnitude as that which was estimated theoretically by Vainshtein for organic crystals with $Z = 6-8$, and having 20-25 atoms in the unit cell. For such a case Vainshtein gives a thickness of about 1200Å as the thickness of crystallites at which kinematic scattering changes to dynamic scattering. From this, we can infer that the sample we have used had a thickness which is beginning to or is showing effects of dynamical scattering.

From Eq. 1002, it is obvious that the purely kinematical case would mean a zero slope, i.e., $m = 0$. The more dynamical the situation is, the larger is the value of $m$. The conclusion
that our sample is on the border line of showing dynamical effect is evident from Figure 1002 and 1003. A "best fit" straight line through the points, as judged by eye, has almost a zero but slightly positive slope. The scatter of the points makes an accurate determination of m almost impossible.

This way of calculating the mean thickness and interpreting the extent of dynamical scattering has validity only if the following set of conditions apply:

1. The two beam dynamical approximation is valid. However, the large intensities in the forbidden reflections may indicate the presence of multiple beam effects.

2. The crystal is of "medium" or intermediate thickness so that absolute dynamical effect is not taking place.

3. The standard structure or reference from which the $\Phi_{cal}$ is calculated, is correct with a high degree of confidence.

4. $\frac{e^*}{N} |\Phi| < 2$.

5. The thickness of each mosaic element must be the same, and there must not be large statistical variations in volume between the individual mosaic elements that give rise to the individual reflections.

The above conditions seem to apply in this case.

The inclusion of hydrogen atoms into the calculation of structural amplitudes produces slightly different values for
Curve to show dynamical effects. $\phi_{\text{cal}}$ is calculated without H atoms. The ordinate is the ratio $I_{\text{exp}}/\phi_{\text{cal}}^2$ which is assumed to be equal to $\sqrt{-\log(K_{\text{exp}})}$ according to the two beam dynamical theory. A best-fit straight line is drawn through these points by eye. Because of the functional form of $K$ (see text), the points corresponding to large $\phi$ values are given more weight than those corresponding to small $\phi$, in drawing the line. The slope of the line determines the extent of dynamical scattering.

Curve to show dynamical effect, $\phi_{\text{cal}}$ is calculated with hydrogen atoms.
Figure 1002.
Graphic representation of Figure 1002.
Graphic representation of Figure 1003.
the calculated structure amplitudes (Appendix B-2). Figure 1003 is the same as Figure 1002 but calculated with the structure amplitudes which includes hydrogen. It is not appreciably changed, hence the mean crystal thickness can be taken as being the same order of magnitude as that which was estimated from the previous plot, particularly since the whole method is extremely crude to begin with.
XI. CONCLUSIONS AND SUMMARY

(1) Patterson Function

All three projections of the Patterson function (h0l, hk0, and 0kl) are qualitatively similar to the "point" Patterson maps calculated from the atom coordinates determined by x-ray diffraction methods. This can be taken to mean that either the Patterson function is not sensitive enough to show the difference between kinematic scattering and dynamical scattering behavior, or that the specimen under investigation is thin enough so that the mode of scattering is totally within the kinematic domain. It has been shown theoretically by Cowley and Moodie [45] that the Patterson function is indeed not sensitive enough to reflect the difference between kinematic scattering and moderate dynamical scattering for light atoms.

(2) Dynamical Scattering

Assuming the validity of the atom coordinates as determined by x-ray diffraction, and assuming the validity of the two beam dynamical scattering theory, a test was applied to the electron diffraction data for the two cases of including and excluding the hydrogen atoms, in order to further assess the extent of the dynamical effect. The results for both cases indicate that the specimen is weakly dynamical with a
roughly measured mean thickness of approximately 1300Å.
This mean thickness is of the same order of magnitude which
Vainshtein calculated, for an organic crystal of 20-30 atoms
in the unit cell, at which kinematic scattering changes into
dynamical scattering effects. To correct the effect of
dynamical scattering, experiments of the type performed by
Honjo, et al., should be attempted in future work.

(3) Hydrogen Atoms
The difference Fourier synthesis shows distinct peaks
located at approximately where the hydrogen atoms should be.
This fact shows that hydrogen atoms have a much more pronounced
effect in electron scattering than in x-ray scattering. Therefore, in future work, the positions of the hydrogen atoms
should be included into the structural amplitude calculation
at an earlier stage than it would be for x-ray work. This
confirms the work of Vainshtein that H atoms can be located
accurately even if the data are not good enough to do the
entire structure.

The difference Fourier map also suggested refinement by
putting in anisotropic temperature factor for the atoms O1
and Cl.

(4) Least Square Method
The structural determination program using the Least
Square approximation method is very sensitive in the present
work to the initial trial structure. With slight variations
in input, it can converge to different minima. This reduces the level of confidence which can be placed on a calculated structure. Again, better statistics in the overdetermination of the parameters may be able to reduce the number of minima.

(5) **Reliability Factor**

The reliability factor \( R \) of the calculated structure is too high. Therefore, further refinement at this stage is not warranted. A high \( R \) value can in the general case be due to a multitude of reasons, such as

a. An incorrect trial structure;
b. Inadequate non-linearity correction;
c. Insufficient overdetermination of the parameters because of the small amount of data available;
d. Dynamical scattering.

It is assumed in this case that the trial structure as determined by x-ray diffraction is correct except for possible dynamical effects which are not significant in x-ray diffraction. Non-linearity correction was discussed extensively in Chapter VI. It is assumed that in the present experiment the necessary correction has been made. The result from Iitaka's work seems to indicate that about 100 unique data points is sufficient to determine the structure even though using a tilting stage to collect reflections from other planes will yield many more data points for a better determination of the parameters.
Hence it may be concluded that the high R value is due primarily to the effect of dynamical scattering that is discussed in point (2) above.

(6) Data Handling

The data collection and conversion system is too limited to process the large amounts of data which any attempt of accuracy in structural determination must require. An automated and integrated system such as the one being developed now (Chapter V) is very much needed.

It has not been the goal of this research to solve completely the crystallographic structure of glycine by the electron diffraction method. This is not yet possible because of theoretical limitations and because the present level of instrumentation is still too crude to yield the large quantity of data that is necessary for very reliable results. Throughout the research, the structure of β glycine as determined by x-ray diffraction has served both as a guide as well as a standard. It is assumed that the structure as "seen" by x-rays is the same as that which is "seen" by electrons. With these assumptions and within the limitations of instrumentation, the above conclusions are drawn from the present investigation.
REFERENCES


5. Vainshtein, ref. 3, p. 4.


42. Vainshtein, ref. 3, p. 162.

43. Vainshtein, ref. 3, p. 172.

44. Schiff, L. I. (1956) Physical Review. 103, #2, 443.


47. Vainshtein, ref. 3, p. 278.


53. Vainshtein, ref. 3, p. 175.

APPENDIX A

Table of the atomic scattering factor $f_{el}$ in p units, i.e., scattering factor relationship to scattering factor or proton for the atoms H, C, N, and O.* Values have been converted into p units from the table given by the *International Tables of x-ray Crystallography*, Vol. 3, 1966.

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<th>H</th>
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*The p unit is defined as the ratio of $f_{el}$ of an atom to the scattering power of a proton scattering at a $\frac{\sin \theta}{\lambda} = 0.1 \times 10^{-8}$. 
APPENDIX B-1

These are the listings of the computer printouts of the first cycle and fifth cycle of the program LSLONG. Here the F's are the same as the notation $\phi$ that was used throughout, which denotes the structural amplitude. The scaling factor $k$ of the $F_{obs}$ is arbitrary in the first cycle, and the reflection $F_{obs} (0,0,0)$ is not an experimental point but a value put in by the program. In the listing, the $H$, $K$, $L$ are the Miller indices, the sin$\alpha$ and Cos$\alpha$ are the imaginary and real parts of the phase of the calculated structure. The other listings are only meaningful to the mechanics of the program and not to the physics of the problem.
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**Notes:**

- Parameters 1 to 20 correspond to specific measurements or calculations.
- The `EST.` column likely represents estimated values.
- The `LAST SHIFT` column indicates the last recorded shift.
- The `SCALE` column seems to indicate a scaling factor or range for each parameter.
APPENDIX B-2

These are the listings of the first and fifth cycles of the program LSLONG when hydrogen atoms are included into the calculation but not refined.

The notations are the same as described in Appendix B-1.
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ACKNOWLEDGMENTS

Special appreciation is expressed to Dr. Robert Glaeser who accepted the student and suggested the problem and to Drs. Cornelius Tobias and Gareth Thomas who, as members of the thesis committee offered many valuable suggestions and comments. I would also like to thank Dr. Peter Fisher for his patient and careful reading of the manuscript and for his enlightening comments, and to Dr. Allan Zalkin whose programs I have used and whose comments I have found helpful. The suggestions and collaboration of many others have contributed substantially to my efforts. In particular, I wish to thank Miss Susan Daniels, Mr. Edward Dowling, Mr. John C. Lee, Mr. Terrance Owyang, Mr. Siddharth Ray, Mrs. Helena Ruben, Mr. Arnold Steinman, Miss Ka Yee Shi, Mr. Frank Upham and Mr. Alfred Windsor for their assistance. The facilities provided by the Lawrence Radiation Laboratory greatly aided my research. I would also thank Mrs. Julia Hess for her expert typing.

This research was supported in part by the Biophysics Training Grant of the National Institute of Health, Grant No. 5 T01 GM00829.
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