

ABSTRACT OF THESIS

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Title of Thesis The Structure of the Sugar Phosphates by X-Ray Methods.

The structure of α -Glucose-1-Phosphate, the Cori Ester, in the form of its dipotassium salt dihydrate has been determined and the structure compared with those of analogous compounds.

The methods used were based mainly on the three-dimensional vector function, partly using the isomorphous diammonium-Glucose-1-Phosphate dihydrate. The information derived from the short intramolecular vectors appearing round the origin of the 'sharpened' vector map was found to be particularly valuable. Direct methods of sign determination were also examined and found to be potentially useful.

Refinement was carried out initially by Fourier methods and finally by the method of Least Squares, all such calculations being done on the D.E.U.C.E. Computer of Glasgow University.

The molecular structure is confirmed as being that found by purely chemical methods. The Glucose-1-Phosphate molecule possesses the long (1.59 Å) phosphorous-ester oxygen bond which is found in other sugar phosphate structures and which seems to be characteristic of this class of compound. In addition, the bond from carbon (1) to the ester oxygen is shorter (1.37 Å) than average. The angle at this oxygen, 124° , is rather wide.

The crystal structure itself is held together by a comprehensive system of non-covalent bonds linking the cations, the free oxygens of the phosphate group, the hydroxyl groups of the sugar ring, and the water molecules. The completeness of this system explains the comparative hardness of the crystal and its lack of any preferred cleavage.

THE STRUCTURE OF THE SUGAR PHOSPHATES
BY X-RAY METHODS.

Thesis presented to the University of Edinburgh
for the degree of Ph.D.
by
Gordon H. Maconochie.



March, 1964.

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INTRODUCTION.

In all living systems, both plant and animal, the phosphate esters of the carbohydrates play roles whose importance is equalled or exceeded only by the proteins and polysaccharides. While one cannot make any rigorous and simple divisions among these esters, as to the relation between structure and biochemical function, it could be said that the pentose esters and the hexose esters do in general, act as functionally different agents, and the introduction will therefore treat them separately.

The pentose esters occur primarily in a directive role, always combined with heterocyclic residues. They may occur as simple monomers or dimers, as in some co-enzymes, or as long chain polymers, as in deoxyribose nucleic acid (D.N.A.) or ribose nucleic acid (R.N.A.). In the former case, the enzyme and co-enzyme will catalyse a particular reaction, given the correct substrate, and in the latter case, it is believed that D.N.A. is the "messenger" for complete sets of genetic instructions, while R.N.A. has the more limited task of guiding syntheses of specific proteins, including enzymes. One well-known exception to the above generalisation is adenosine-5'-triphosphate (A.T.P.), a pentose ester which is the phosphorylating agent in many biochemical reactions. It is able to transfer one of its two terminal phosphate groups to a number of different molecules, without showing any particular specificity.

The /

The structures of only relatively few of these compounds have been fully worked out by X-Ray methods, and a summary of the results is given diagrammatically for Cytidine-3-Phosphate (Fig. 1, Ref. 8), Adenosine-5'-Monophosphate (Fig. 2 Ref. 9) and Calcium Thymidylate (Fig. 4, Ref. 11). The structure of Aminoethanol Phosphate (Fig. 3, Ref. 10) has been included because of the accuracy to which the phosphate ester group has been refined. Barium-Ribose-5-Phosphate (Ref. 12) has also been solved and refined to moderate accuracy.

The methods used are discussed later, along with those used in the solution of Glucose-1-Phosphate. Part of the reason why so few have been studied lies in the difficulty of obtaining suitable crystals, part in the inherent difficulties of solving many-atom structures of low symmetry. However, it may be noted that the general structure of D.N.A. was deduced largely from models, built up from accurate molecular structures of the few simple related groups which had been studied at that time, such as Adenine, Guanine, Cytosine, and the furanose rings occurring in Ribose and Sucrose. Possibly the least satisfactory part was the phosphate link, whose structure could only be approximated to on geometrical considerations. The hydrogen bonding system, which imparts to these molecules their unique capacity for self-reproduction could not have been more than a guess, had it not been that the sub-units of the component nucleotides were known with sufficient accuracy to permit the assessment of distances between heterocyclic rings in the double helix /

Fig 1

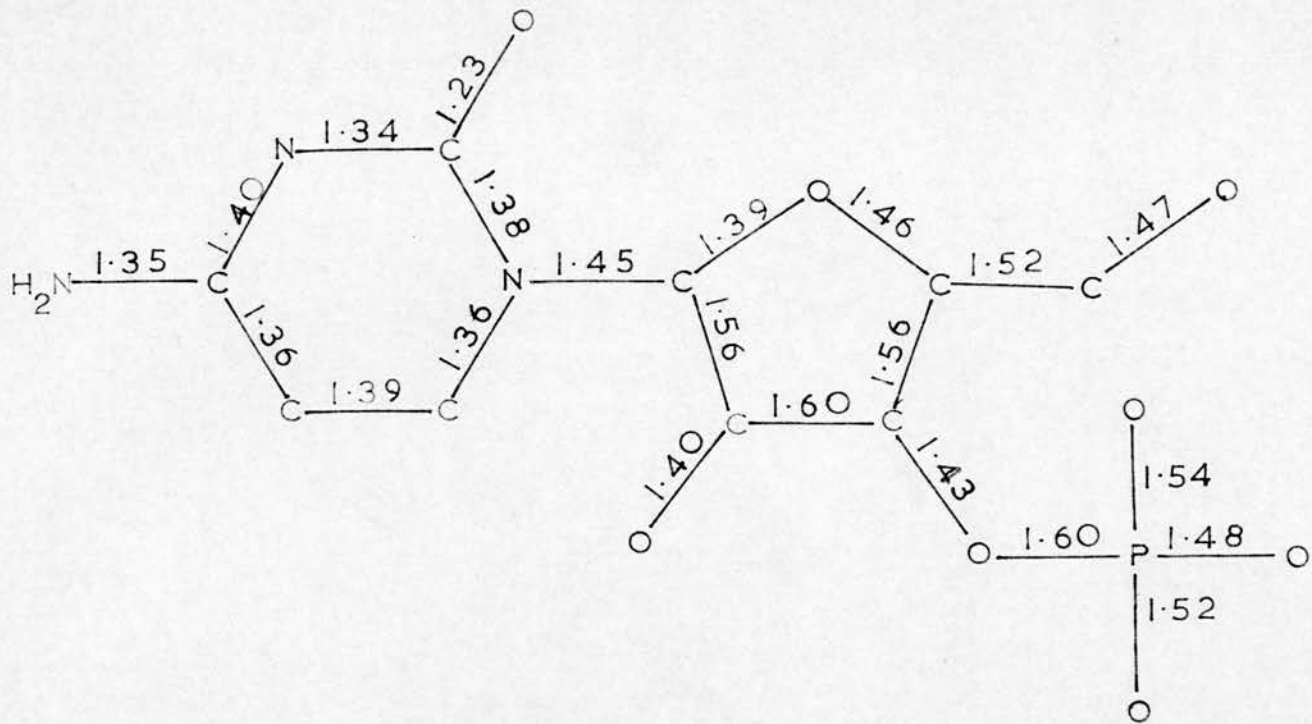


Fig 2

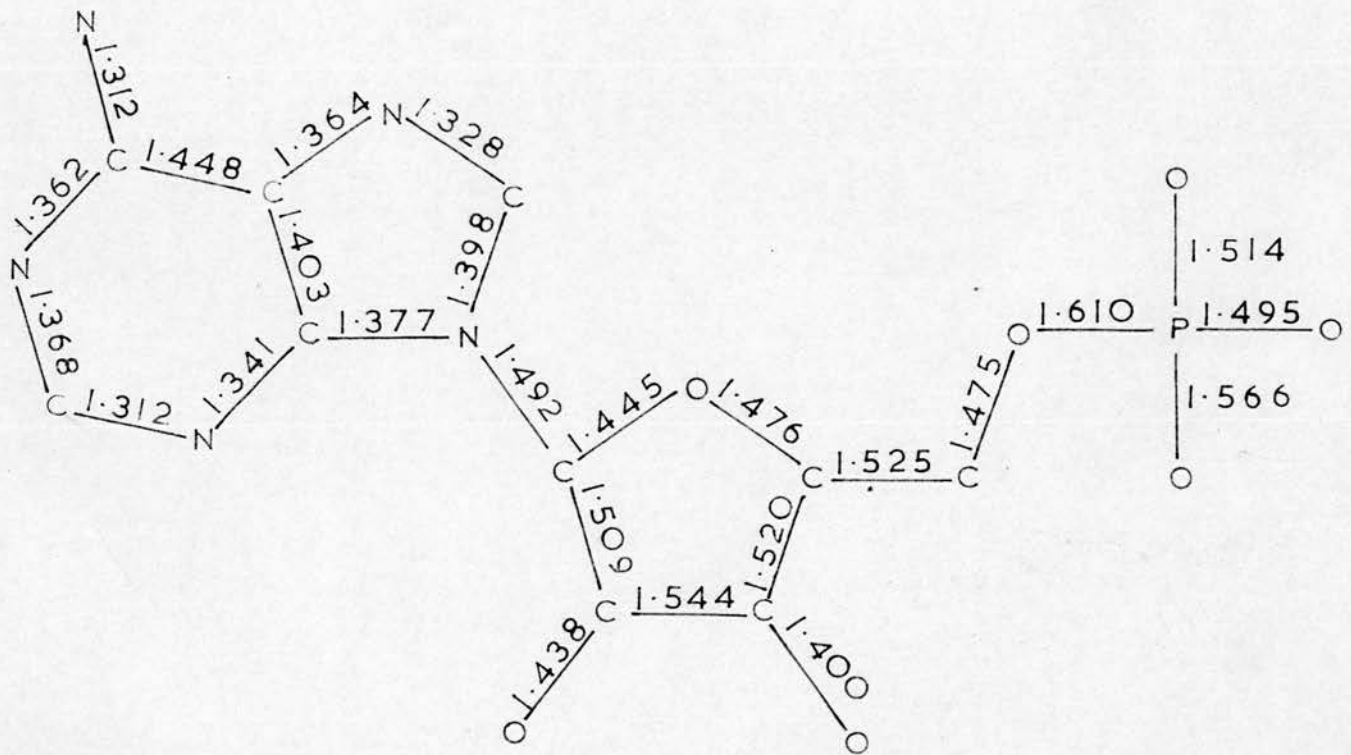


Fig 3

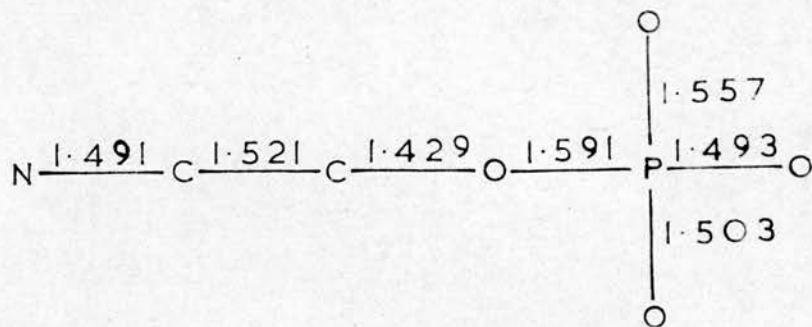
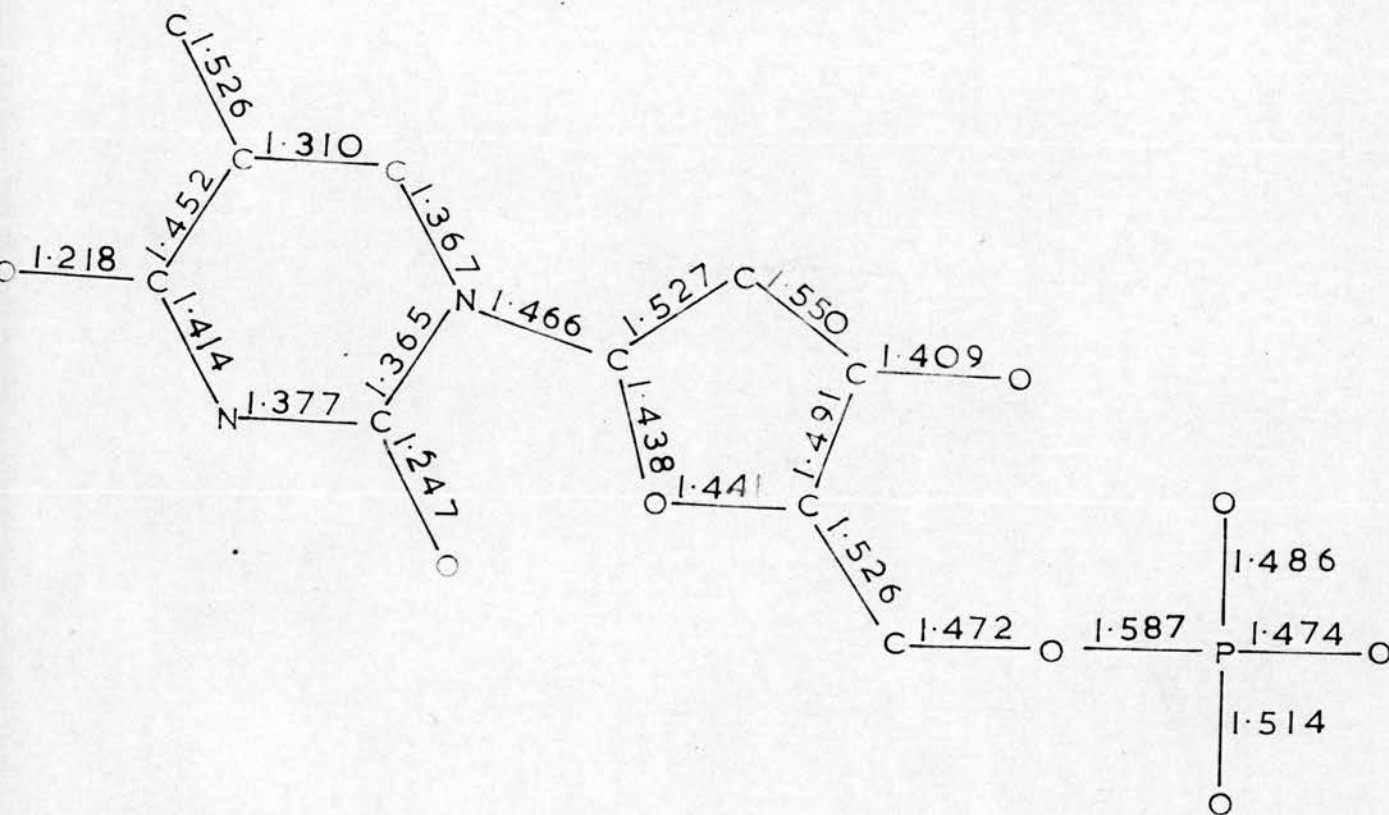


Fig 4



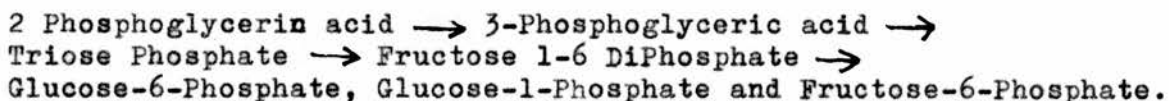
helix, and in particular to show that only certain pairings were possible. (Refs. 1, 4, 5, 7, 13).

Hexose Esters.

The hexose esters, by contrast, are not concerned in any directive function. Polymerised hexose phosphates have only been observed fairly recently in the material forming the cell walls of certain micro-organisms, their role being apparently entirely structural. These have been named the Teichoic acids (Refs. 14, 15).

Fructose 1-6 Diphosphate, Fructose-6-Phosphate, Glucose-1-Phosphate and Glucose-6-Phosphate account for the main part of the observed esters, these being most in evidence in fermentation, respiratory and photosynthetic reactions. In the last of these, simpler glycerophosphates and triose phosphates precede the appearance of the hexoses.

One tentative chain of conversions given (2) includes the sequence:



Glucose-1-Phosphate is the immediate precursor of starch.

Possibly even more interesting from the point of view of the difference between Hexose and Pentose esters is the conversion of Glucose-6-Phosphate to Ribose-5-Phosphate, a constituent of R.N.A. (Ref. 1. p.191)

The biochemist Lipmann has suggested that in synthetic and /

and degradative reactions, the elements of phosphoric acid may be visualised as replacing those of water. The energetics of the various transformations involved are much more favourable than would be the case with the non-phosphorylated sugars. (Ref. 13). Typically, a biochemical process may start with a carbohydrate which is then phosphorylated by A.T.P. or another 'high energy' phosphate, under the catalytic action of the appropriate enzyme, undergoes conversions, such as the chain listed above, and finally gives some product, e.g. glycogen or starch, with loss of the phosphate group.

In this context, it may be worth commenting on the 'high energy phosphate' theory of Lipmann. Briefly, this suggests that certain phosphate bonds act as intermediate energy stores for biochemical reactions. A.T.P. is the best known of these, though far from being the only one. The distinction is made on the basis of the heats of hydrolysis of the phosphate bond (Ref. 2. p. 209) thus:

<u>Phosphate</u>	<u>Heat of Hydrolysis</u> <u>per Phosphate Group.</u>
Glucose-6-Phosphate	2,500 cal./mole
Glucose-1-Phosphate	4,800 cal./mole
Fructose-1-6-Diphosphate	2,500 cal./mole
Adenosine-5'-Monophosphate	2,500 cal./mole
Adenosine-5'-Diphosphate	12,000 cal./mole
Adenosine-5'-Triphosphate	12,000 cal./mole

The difference between the terminal phosphates and that adjacent to the ring in A.T.P., A.D.P. and A.M.P. is most marked.

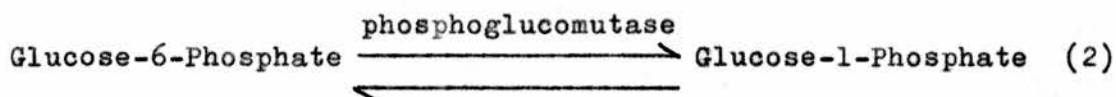
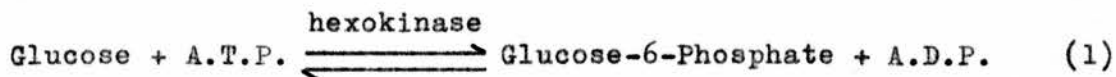
Glucose-6-Phosphate must be converted to the slightly higher energy Glucose-1-Phosphate prior to the formation of Glycogen /

Glycogen, Starch, Sucrose, or other di- or poly-saccharides, since these are linked through the 1-position. This conversion is catalysed by the enzyme Phosphoglucomutase, though the exact mechanism is not very clear.

No 'high energy' phosphate has yet been studied by X-Ray investigation, probably because of the difficulty of crystallising these substances.

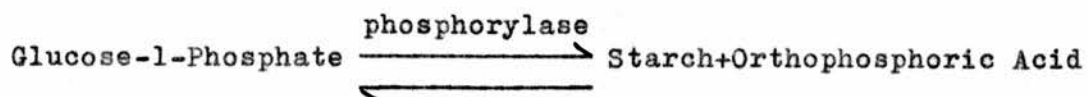
The Structure of Di-Potassium Glucose-1-Phosphate Dihydrate.

General: As with all 1-substituted sugar derivatives, Glucose-1-Phosphate can exist as two anomers, α and β . The molecule studied was the naturally occurring form, known as the Cori Ester, after its discoverer. It is not formed directly, by straightforward esterification of the 1-hydroxy group, but as follows:



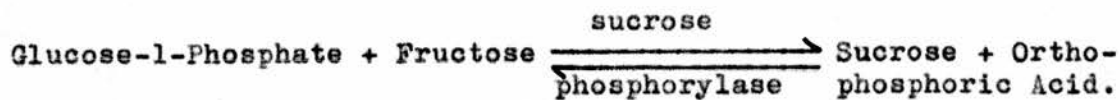
In (1) equilibrium is far to the right, and in (2) well to the left, with about 95% Glucose-6-Phosphate and 5% Glucose-1-Phosphate at equilibrium.

The Glucose-1-Phosphate so formed may then engage in other reactions, the most important of which is probably the synthesis of starch. This process can be summarised by the simple equation.



The energy required to link the Glucose units comes from the hydrolysis of the phosphate bond, hence, ultimately, from the A.T.P. which phosphorylated the Glucose in the first place. Starch cannot be formed directly from Glucose alone. With the Cori Ester and a different enzyme found in animal systems, Glycogen is formed. The production of Sucrose also requires Glucose-1-Phosphate, thus:

Glucose /



Other disaccharides, some quite unknown in nature, can be synthesised by reaction of the appropriate sugar with the Cori Ester in the presence of Sucrose Phosphorylase (Ref. 2).

Wolfson and Fletcher (Ref. 16) describe a chemical study of the structure. The high dextrorotation was interpreted as indicating the α - ^{conformation} configuration. Synthetic Glucose-1-Phosphate was prepared by treatment of α - Acetobromoglucose with Silver Orthophosphate. The resulting material, after hydrolysis of the acetate groups and subsequent purification, was found to be identical with the Cori Ester. The fact that Starch and Glycogen are α -linked and synthesised via the Cori Ester, whereas the β -linked Cellulose is not, might also be evidence for the α -form. None the less, in stereochemical problems, it is difficult to be absolutely certain of conformations deduced by chemical methods alone.

These authors, in passing, point out that Glucose-1-Phosphoric Acid is stronger than Orthophosphoric Acid, and go on to suggest that it might be more accurate to describe it as a glycoside rather than as an ester. This terminology, although probably more correct, does not seem to have been generally accepted.

Experimental Methods.

Glucose-1-Phosphate is available commercially in a very pure state as the Di-Potassium salt Di-hydrate. Crystals were grown of sizes up to 2 x 1 x 1 mm., occasionally bigger, by /

by the slow cooling of an aqueous solution, saturated at 60°C , down to about 25°C . In carrying out this process, the construction of a large, steam-heated water tank, lagged with glass wool, was found to be very useful, as the large quantity of water in it led to much slower cooling than could be obtained by using a vacuum flask. Cooling from about 60°C to about 30°C took some eighteen hours. The crystals were observed to be monoclinic with a length-breadth ratio around 2:1. They did not show an easy cleavage in any direction being, in fact, sufficiently hard to be ground into spheres using Bond's sphere grinder (Ref. 17), or into approximate cylinders by being rolled between sheets of fine emery paper.

A crystal about 0.4mm. in diameter was selected and mounted with the prism axis lying along the axis of rotation. After alignment by means of oscillation photographs, a Weissenberg photograph of the zero-layer was taken. In this and all subsequent work, the source of radiation was a continuously evacuated, self-rectifying Raymax tube. The data actually used in the determination were collected using Cu - K_{α} radiation, and a running voltage and current of 50kV and 20 mA. The camera employed was a 5-cm. radius, vertical travel, normal beam camera (Ref. 18).

Approximate reciprocal lattice dimensions were obtained initially from the $h\ 0\ 0$ and $0\ 0\ l$ reflections. $\sin \theta$ for each reflection was found by measuring its distance on the film from the sharply defined shadow of a knife-edge strip, which is part of the camera, and which subtends an exactly known angle at /

at the axis of rotation of the crystal. On extrapolating the values so found to $\sin^2 \theta = 1$, reasonably good approximations to a^* and b^* were obtained. The cell was monoclinic, and β^* was calculated from the separation of the axial lines on the Weissenberg photograph. These approximate values of a^* , b^* and β^* were then improved in turn, using $h 0 l$ reflections of high $\sin \theta$ and extrapolating to $\sin^2 \theta = 1$, until consistent values were obtained. Zero layer photographs were obtained about the a and c axes, and values of a^* , b^* , c^* and β^* arrived at in the same manner.

The separation of the reflections of $K\alpha_1$ and $K\alpha_2$ radiation at high angles was sufficient to permit measurements of the $\sin \theta$ values to be made on the $K\alpha_1$ spots alone.

The cell dimensions finally obtained, taking the wavelength of $Cu K\alpha_1$ to be 1.5405 Å were:

$$\begin{aligned} a &= 10.440 \text{ Å} \pm 0.005 \text{ Å} \\ b &= 9.025 \text{ Å} \pm 0.005 \text{ Å} \\ c &= 7.518 \text{ Å} \pm 0.005 \text{ Å} \end{aligned}$$

$$\begin{aligned} \alpha &= \gamma = 90^\circ \\ \beta &= 110^\circ 24' \pm 6' \end{aligned}$$

The cell volume = 665 Å³.

The density, found by flotation in a solution of iodomethane and chloroform, is 1.85 gm/cc, the density of the flotation liquid being found by direct measurement of the weight in a density bottle.

On the assumption that there are two structural units per unit cell, the molecular weight of each should be 370.5. The molecular weight of DiPotassium Glucose-1-Phosphate Dihydrate is 372.4, hence there are two units in the cell. The linear absorption /

absorption coefficient, μ , is equal to 80 cm.^{-1} for Cu $K\alpha$ radiation.

The only systematic absences were those occurring in the $0k0$ reflections when $k = 2n + 1$, indicating a screw axis parallel to b . The space group is therefore $P2_1$.

Intensity Measurements.

An obvious problem in the measurement of the diffracted intensities was the rather high absorption coefficient.

Absorption effects can be minimised either by using very small crystals, or by using a short wavelength radiation, absorption coefficients decreasing rapidly with diminishing wavelength.

Lipson and Cochran (Ref. 24) state that, if absorption is to be negligible, the product μt , where t is the mean thickness of the crystal, must be about 0.05. When $\mu = 80\text{cm}^{-1}$ this leads to a crystal diameter around 0.006 mm, which is too small to be practical. Even with crystals around 0.1 mm diameter, it was found that high angle reflections were extremely weak, unless long exposures, comparable with the filament 'life' of a continuously evacuated tube, were employed.

Calculation of the absorption coefficient for Mo K α radiation, of 0.71 Å wavelength, showed it to be only about 10 cm^{-1} . Accordingly, a Molybdenum anode and a zirconium filter were fitted to the X-ray tube. Oscillation photographs were taken, with a fairly large crystal, at a working voltage and current of 70 k V and 7.0 mA. Raising the tube current led to instability. The result diffraction pattern recorded was extremely weak, only a few low-angle reflections being discernable. This was not too surprising, since the absorption of X-rays by the Silver Halide crystals in the film, which leads to blackening, also decreases greatly with decreasing wavelengths. Much more serious was the greatly increased background on the film. This fogging is presumably caused by a greater ratio of 'white' /

'white' to characteristic radiation being emitted from a Molybdenum target, and this method had to be abandoned.

M.J. Buerger has shown that for a cylindrical crystal, the optimum ratio of absorption to diffraction is obtained when the crystal diameter = $2/\mu$. If $\mu = 80 \text{ cm}^{-1}$, one obtains a value of 0.25 mm. Intensity data were therefore obtained from crystals whose cross sections were between 0.2 and 0.3 mm. The absence of an easy cleavage in any particular direction made the use of rather irregularly shaped fragments unavoidable for axes other than the b -axis, which could be ground to an approximate cylinder. Since the hardness appeared to be anisotropic, the cylinders tended to be elliptical in cross section rather than circular. Spherical, or almost spherical, crystals in the correct size range were obtained but the difficulty of orientating these was much greater than with a crystal chip, there being no recognisable edges or faces on them. If it is not possible to get the desired axis vertical with the normal goniometer arcs, it is necessary to demount the crystal, and replace it more nearly at the correct angle. Marking the sphere at the 'north', 'west', and 'east' positions with coloured ink was tried, but the smallness of the crystal necessitated extremely small marks, and these came off too easily in the de-mounting and re-mounting process.

To begin with, only the $0k1$, $1k1$, $h0l$, $h1l$, $hk0$ and $hk1$ layers were photographed, and the intensities of the zero-layer reflections measured. The multiple film technique was employed with one Ilford 'Industrial G' and two 'Industrial R' films. the G-film being nearest to the crystal.

Intensities /

Intensities were measured by visual comparison with a series of oscillation photographs of different timed exposures, recorded on the same types of film, using the same crystal as for the Weissenberg photographs. Scaling between the 'G' and first and second 'B' films was carried out by measuring the intensities of reflections common to two films. A proportional relationship, $I(G) = k_1 \times I(B_1)$ where $I(G)$ = intensity as measured on the G - film and $I(B_1)$ = intensity of the same reflection as measured on the first B - film, was assumed. A similar relation was assumed for the ratios $B_1:B_2$ and $G:B_2$. The 'best' value of k is found then by the relationship $k_1 = \frac{\sum(I(G))^2}{\sum I(G) \times I(B_1)}$ (Ref. 29, p. 335).

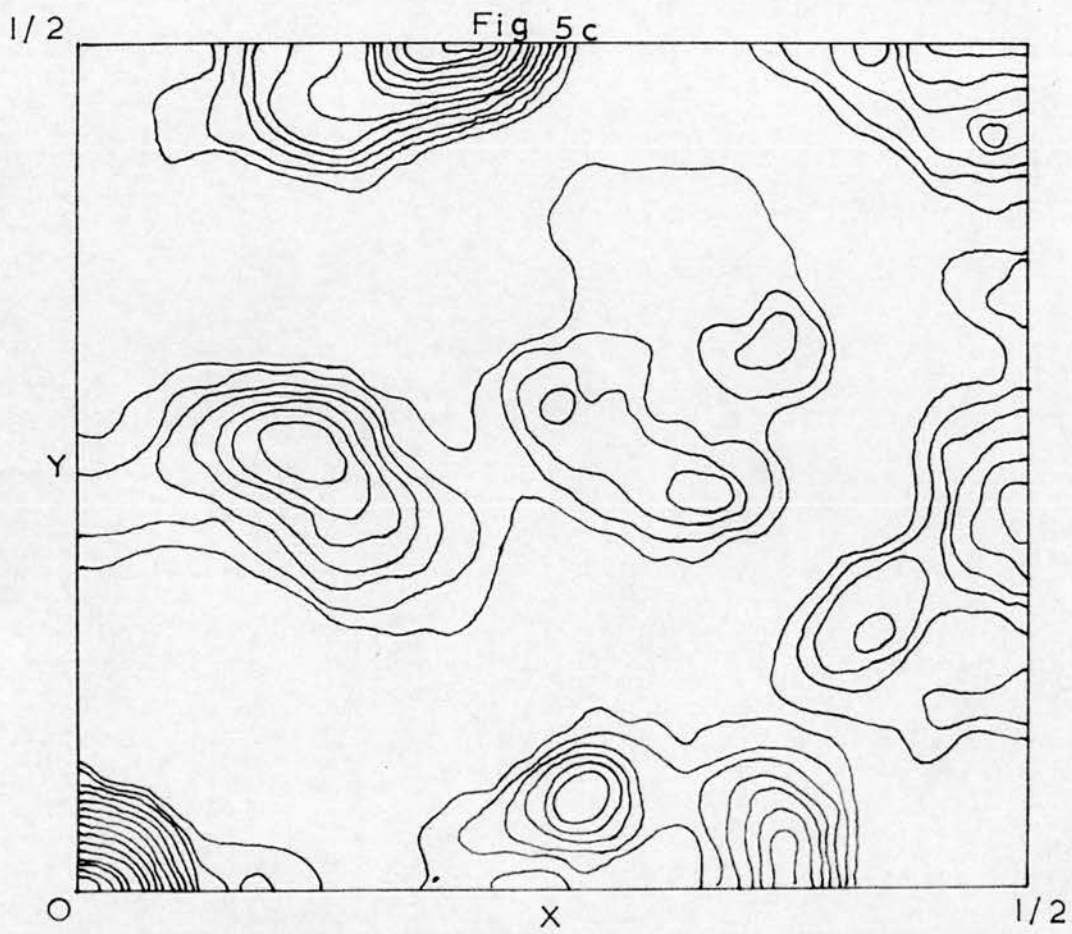
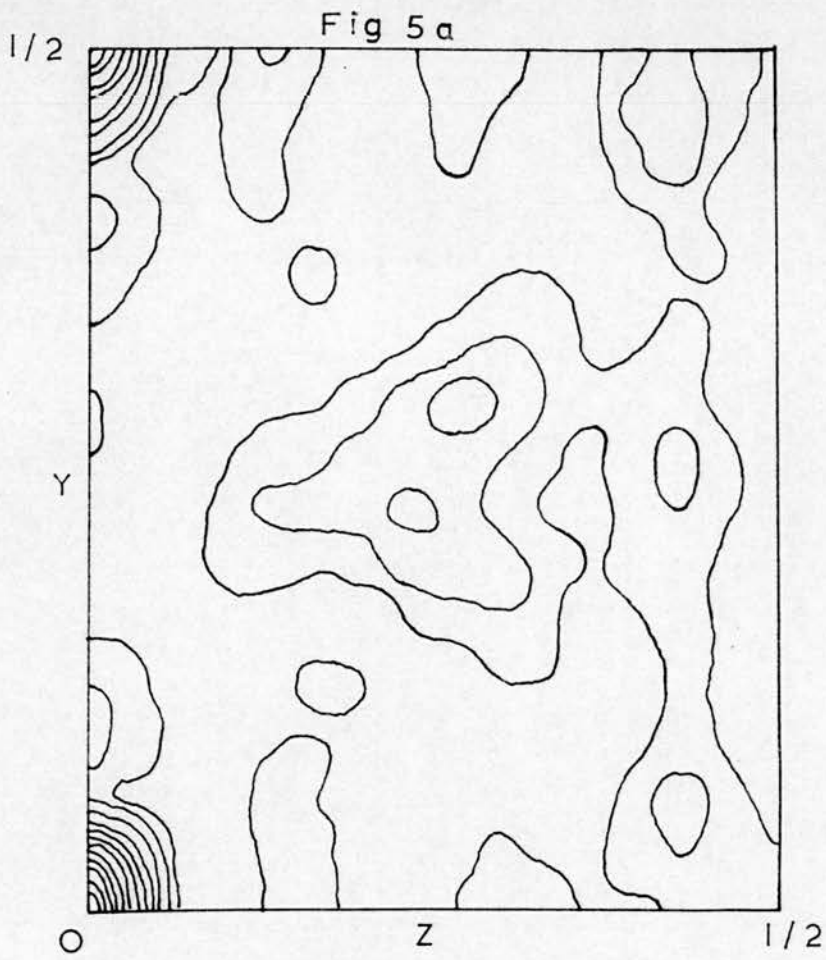
The estimated intensities so obtained were corrected for Lorentz and polarisation factors by the method of Cochran (Ref. 19). The resulting squared structure amplitudes were left on a relative scale. This was also the method used for all succeeding films.

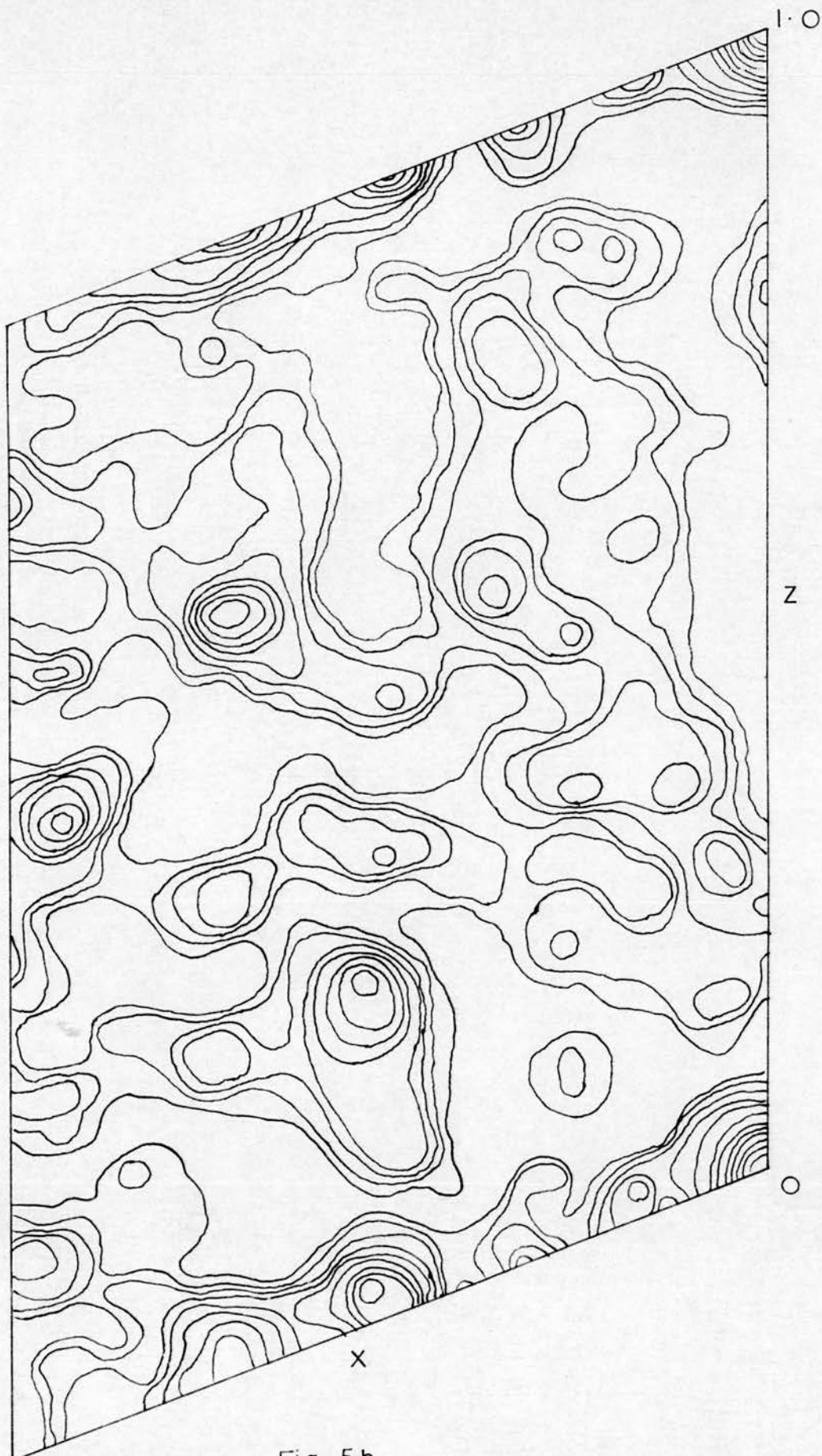
The Patterson Function,

Projections of the Patterson function down the three cell axes were calculated (Ref. 20). 'Sharpened' projections, which give greater resolution of the maxima, were also calculated and, in practice, used in preference to the unsharpened projections. Calculations at this stage were carried out using the Beevers-Lipson strip method, along with an electro-mechanical analogue machine.

Reproductions of the revised a-axis, the b-axis, and the c-axis projections are given in figures 5a, 5b, and 5c, in that order. The most obvious features were the heavy 'ridge' of vectors along the a-axis of the b-axis projection, i.e. along $Z = 0$, and the row of peaks, including one which is exceptionally large, at $y = \frac{1}{2}$ on the c-axis projection. The last mentioned row of peaks was expected, as Harker has pointed out that certain symmetry elements give rise to an accumulation of vectors on certain lines or planes. In this case, we have the symmetry element 2_1 , with the equivalent positions (x, y, z) and $(-x, y + \frac{1}{2}, -z)$ which must give rise to a peak for the i 'th atom, position (x_i, y_i, z_i) in the unit cell, at $(2x_i, \frac{1}{2}, 2y_i)$ in vector space, i.e. all atoms in the cell give rise to a vector somewhere on the plane $y = \frac{1}{2}$. In projections parallel to this plane, the result is an accumulation of peaks on a line at $y = \frac{1}{2}$. In general, if we have two unrelated atoms A and B, at positions (X_A, Y_A, Z_A) and (X_B, Y_B, Z_B) , the twofold screw axis will produce vector peaks at:

(2 /





1/2

Fig 5b

$(2x_A, \frac{1}{2}, 2z_A)$ and $(2x_B, \frac{1}{2}, 2z_B)$ - ("Harker peaks")

$(x_A - x_B, y_A - y_B, z_A - z_B)$ and $(x_B - x_A, y_B - y_A, z_B - z_A)$ ("Difference peaks")

$(x_A + x_B, y_A - y_B - \frac{1}{2}, z_A + z_B)$ and $(x_B + x_A, y_B - y_A - \frac{1}{2}, z_B + z_A)$ ("Sum peaks")

In attempting to locate the sugar part of the compound, use was made of the symmetry properties of the pyranose ring. While the complete Glucose-1-Phosphate anion does not possess a centre of symmetry, certain parts of it do. All the ring atoms, plus the oxygen atom attached to C_2 , and the atom C_6 , are related by a centre of symmetry in the middle of the ring. When the space group has a centre of symmetry or a centrosymmetric projection, this causes the Patterson function to have a fairly large multiple peak at a point whose vector separation from the origin is equal to that of the centrosymmetric, or approximately centrosymmetric, units in the cell. Where only ^{one} centrosymmetric projection exists, the separation in this projection only can be found. This is the 'centre-centre' peak, and the correct identification of this is sufficient to locate the molecular centre.

Further, when the molecule contains a number of vectors which are parallel to each other, as is the case with the pyranose ring, a characteristic pattern of maxima in the vicinity of the Patterson origin, at a radius of from 1.5 to 2.5 Å, will be produced. A specific orientation of the molecule will give rise to a corresponding orientation of this pattern. It is not necessary that the space group should have a centre of symmetry to utilise this fact. (Ref. 21)

If /

If the centre-centre vector and the multiple intramolecular vectors are correctly identified, this is sufficient to locate the position and orientation of the symmetrical or partially symmetrical molecule in the unit cell. The overall strategy in approaching the problem, then, was to find positions for the K^+ and P atoms, these being rather heavier than the others, which gave suitable Harker, 'sum' and 'difference' peaks, and to deduce the position and orientation of the Glucose ring from the selected centre-centre and multiple peaks.

Unfortunately, the amount of overlapping of maxima in projection is so considerable that it is not always possible to detect the required peaks. This is especially true when there are atoms in the cell which are somewhat, but not greatly, heavier than the ring atoms. A single medium-medium vector will then be of about the same weight as the multiple light-light vectors. It was also true in this case that the centre-centre vector would only occur as a large multiple peak on the **b**-axis projection, as this is the only projection which is centrosymmetric. On other projections, the vectors giving rise to the 'centre-centre' peak will appear as a patch of 'positive ground' stretched in the direction of the b-axis, the degree of diffuseness depending on the angle between the molecular plane and this axis. Finally, if Patterson projections are sharpened, the area around the origin is considerably affected by series termination errors. Since some of the intramolecular vectors are short, about 1.5 \AA , this means that they will occur in a region containing peaks and troughs not actually attributable to real /

real interatomic vectors. If the Patterson projection is not sharpened, the maxima are so coalesced as to be beyond interpretation.

Initially, only the positions of the medium weight atoms were sought. From the b -axis Patterson, it was assumed that these three atoms were collinear, and parallel to 'a'. When what seemed to be a reasonable 'fit' on the Patterson map has been obtained, $h\ 0\ l$ structure factors were calculated and compared with the observed values. A considerable amount of time was spent trying to obtain a set of positions which gave some agreement between observed and calculated structure factors, but no success was obtained.

In view of the complete lack of progress, it was decided that the three dimensional Patterson function might be more fruitful, and that a salt of Glucose-1-Phosphoric Acid which was isomorphous with the Potassium salt would also be useful. Rubidium and Ammonium salts are often isomorphous with the equivalent Potassium salt, and these derivatives of Glucose-1-Phosphoric Acid were therefore prepared. This was done by passing a solution of the Di potassium salt through a resin ion exchange column in the Hydrogen form, (Amberlite I.R. 120 H), thereby obtaining the free acid, and neutralising with Rubidium Carbonate or Ammonia, as required. One difficulty with this procedure is that the Cori Ester is labile to acid, with a consequent risk of self-hydrolysis. Both salts were prepared successfully, though not, perhaps, in a very pure state. The ammonium salt did not crystallise, possibly because of impurity. The Rubidium salt, however, crystallised well, and Weissenberg photographs about two axes were obtained.

These /

These showed it to possess an orthorhombic cell, the space group being probably $P2_1^2 2_1 2_1$. Since it was not isomorphous, this derivative was abandoned, while efforts to crystallise the ammonium salt were continued.

Concurrently the general $h k l$ intensities for the dipotassium salt were measured. These were estimated on the earlier okl , $1kl$, hol , $h1l$, hko , and $hk1$ layer photographs, supplemented by upper-layer normal-beam Weissenberg photographs taken about the c , b , and what appeared to be the a -axis. Scaling was carried out by comparing reflections common to two or more axes. During the scaling process, it became obvious that the " a " axis Weissenbergs, from $2kl$ onwards, were not consistent with the other films. A careful comparison of the intensities with those on the non a -axis films, and calculation of the lengths of the cell diagonals showed that the short $a - c$ diagonal was similar in length to the a -axis. A cell with this diagonal as the longer non-unique axis would have the dimensions

$$\begin{aligned} a^1 &= 10.50 \text{ \AA} \\ b &= 9.025 \text{ \AA} \\ c &= 7.518 \text{ \AA} \\ \beta^1 &= 111^\circ 44' \end{aligned}$$

the original cell being, as given before:

$$\begin{aligned} a &= 10.44 \text{ \AA} \\ b &= 9.025 \text{ \AA} \\ c &= 7.518 \text{ \AA} \\ \beta &= 110^\circ 24' \end{aligned}$$

It is apparent that these two cells have rather similar dimensions. This similarity between the two possible cells also intruded at a later stage in the determination. After re-indexing the films, consistent /

consistent F^2 s were obtained.

To begin with, only the unsharpened three-dimensional Patterson function was calculated. This was decided on firstly because it was assumed that resolution would be sufficiently improved over the two-dimensional case, even without sharpening, and secondly, because of doubts about the accuracy of high $\sin \theta$ intensities. These appeared mainly on upper-layer photographs, on which reflections were either elongated or compressed, making them more difficult to estimate. This weighted against the exaggeration of these terms relative to the lower angle reflections.

The calculation was carried out on the Glasgow University D.E.U.C.E. Computed, using Fourier summation programme of J.S. Rollett. Sections were calculated in planes perpendicular to the b-axis at intervals of $1/40$ th of b . Each plane was sampled at intervals of $1/40$ th of 'a' and $1/30$ th of c. The sections were contoured and drawn out on glass plates which were then arranged at the correct separation in a frame lit from below. This enabled one to get an overall qualitative view of the Patterson function in three dimensions. For more detailed work, each section was examined individually.

The most obvious features of the Patterson were heavy peaks along $z = 0$ at both $y = 0$ and $y = \frac{1}{2}b$. It was also plain from even a superficial inspection that the a-axis projection, which had been calculated by hand, was incompatible with the three dimensional synthesis.

The calculation was checked and it was found that a projected/

projected area of $\frac{1}{2}c$ by $\frac{1}{4}b$ had been calculated instead of $\frac{1}{2}c$ by $\frac{1}{2}b$. This was rectified, and the corrected version is shown in figure 5a. Both this projection and the three-dimensional synthesis strongly suggested that the pyranose ring was lying with its plane perpendicular to the screw axis, and one of the long diameters parallel to the a-axis. Since the medium weight atoms were also lying in a common plane, it followed that the vectors from these atoms to the pyranose ring must also be lying in a plane, again perpendicular to the screw axis. This agreed very well with the observation that the only regions of marked vector density apart from $y = 0$ and $y = \frac{1}{2}b$ was the plane $y = \frac{11}{40}b$. From the foregoing evidence, it was concluded that the medium weight atoms lay almost in a plane perpendicular to the screw axis, and that the sugar ring lay in a plane parallel to this and separated from it by about $\frac{11}{40}b$, some 2.5 Å. This general picture of the structure proved to be correct.

Some idea of the conformation could be gained from the observation that if the β -Glucose-1-Phosphate molecule is arranged so that the Phosphorus atom is 2.5 Å from the ring plane, the oxygen atoms of the phosphate group are compelled to approach the ring atoms closely. The α -form, however, is in its most ~~extended~~ extended configuration when the phosphorus atom is 2.5 Å from the ring plane, thus strongly tending to confirm the chemical evidence for the α -conformation.

The grouping of the atoms in planes has the marked disadvantage that it leads to a heavy concentration of peaks on the Harker section, since both Harker and non-Harker vectors will occur at and near $y = \frac{1}{2}b$. From the orientation of the molecule, it /

it was apparent that the 'centre-centre' vector must lie distributed about 0.5 \AA , which is the depth of a pyranose ring, above and below the Harker section. This deduction, while correct, led to a mis-identification of certain peaks, that is, some peaks at about $y = \frac{1}{2}b \pm 0.5 \text{ \AA}$ were given the role of 'possible centre-centre peaks'. Other possibilities were correspondingly weighted less heavily. In retrospect, this misinterpretation was probably the largest single obstacle requiring to be removed before obtaining a refinable trial structure.

Since a structure giving satisfactory Harker, sum, difference, and centre-centre peaks could not be devised, work was temporarily concentrated on the a-axis projection, for which the x-co-ordinates of the atoms were not required. A fairly prominent peak at the appropriate y-co-ordinate was selected as the 'centre-centre' peak, and from this and the known molecular orientation, y and z parameters for the sugar ring were obtained. The y co-ordinate of one atom can be set arbitrarily at zero, with space group $P2_1$, and since K_1 , K_2 and P were believed to be collinear and at the same y-level, all three were placed at $(x,0,0)$ i.e. at the origin in the a-axis projection. Agreement between calculated and observed structure factors was reasonably satisfactory. Refinement was carried out by means of $(F_{ob} - F_{calc})$ Fourier synthesis. In the course of refinement, the entire molecule moved in the direction of an increasing z-co-ordinate, indicating that the originally chosen centre-centre peak was incorrect. Only very small changes in molecular orientation were required. Peaks in the $(\rho - \rho_z)$ map /

map were taken as being the water molecules, as there were only two of them, and these were included after the fourth cycle. The structure refined to a value of 'R' (i.e. $\frac{\sum |F_o| - |F_c|}{\sum |F_o|}$) of 0.31, but would not go below this somewhat high value. Fluctuations in the ($\rho_o - \rho_c$) map. at this stage, were considerable but gave no indication of what atomic shifts were required, since peaks and hollows occurred in spaces where there were no atoms. The area around (x,0,0) was at a level of almost zero, and more or less flat, reinforcing the belief that at least the medium weight atoms were correctly placed. Attempts were now made to find a structure which would fit the three-dimensional Patterson, retaining the y and z co-ordinates found from the a-axis projection. This was done by examining the sections from $y = \frac{9}{40}$ to $y = \frac{11}{40}$, and, on the correct assumption that these sections contained the medium atom \longrightarrow light atom vectors, and hence would contain three 'images' (Ref. 22) of the sugar ring, separated by the same distances as the heavy atoms, trying to 'fit' three molecules on to the peaks in these sections. The z-parameter of the molecular centre was kept constant, and moves made only in the x-direction. Since the a-axis is 10.44 Å long, and the 'heavy' atoms had to be at reasonable distances from each other, a separation of about 3.5 Å between images was required. None of the structures postulated from this procedure gave really convincing vector distributions, and those for which structure factors were calculated showed no correlation with observed values.

The Patterson function was now recalculated with a slightly increased number of reflections, about 1,350 instead of 1,200 /

1,200, and with sharpening. The sharpening function was obtained by dividing the F^2 's in the $h\ 0\ l$ plane into ranges of $\sin^2\theta$ containing nearly equal numbers of reflections and calculating the factor required to bring the average F^2 in each range up to that of the innermost range. This, in effect, converts the real, diffuse atoms into imaginary, point scatterers. The limitation of a finite number of terms being available makes reduction to a perfect point impossible, but the volume of each peak is, none the less, greatly reduced. The function $(1 + 8 \sin^3\theta)$ was found to be a fairly good fit through the factors required, and this was accordingly applied to the data. Although resolution of the peaks was considerably improved, no new interpretation of the Patterson was arrived at.

The Ammonium salt had not yet been crystallised, and after concluding that the methods employed up to this point were not succeeding, it was decided that a completely different approach should be tried.

Direct Methods.

By 'direct methods' one implies methods by which the phases of structure factors are deduced from mathematical relationships between their amplitudes. In practice, only centrosymmetric space groups or plane groups may be solved, as the relationships at present known are effective only when applied to structure factors with a phase angle of 0 or π radians; i.e. a sign of (+) or (-). The space group $P2_1$ is non-centrosymmetric and possesses only one centrosymmetric projection, hence only the $h\ 0\ l$ structure factors can have their phases determined, even in principle.

In order to apply direct methods, it is convenient to convert the observed structure factors into unitary structure factors. This is defined as the ratio of observed absolute amplitude to maximum possible amplitude. It is given the symbol 'U' and must be between -1 and +1. The procedure determining U is as follows:

If f_j is the scattering factor for a particular atomic type at a particular $\sin \theta$ value, and $\sum_{j=1}^N f_j$ denotes the sum over all atoms in the cell, then n_j , the unitary scattering factor of the j 'th atom is equal to $f_j / \sum_{j=1}^N f_j$.

The quantity ϵ is defined as:

$$\epsilon = \sum_{j=1}^N (n_j)^2, \text{ and can always be calculated from the}$$

theoretical scattering curves for each type of atom present.

F^2 , if its value were already known on an absolute scale, could readily be converted to U. Since F^2 is not known absolutely, U must be found by a statistical method similar to that/

that of Wilson (Ref. 26). He showed that, taken over a number of reflections in the same $\sin \theta$ range, the average $|F|^2$ was equal to $\sum_{j=1}^N f_j^2$. If we designate the average $|F|^2$ in a particular $\sin \theta$ range as $\langle I \rangle$, then by plotting $\epsilon / \langle I \rangle$ against $\sin \theta$, a curve is obtained of the square of the correcting ratios required to convert F's to U's. Let this be called ϕ . By multiplying the observed F's on the reciprocal net by $\sqrt{\phi}$ for the appropriate $\sin \theta$ value, U's were obtained. A table of U x 1000's is given (Table 1).

The simplest, and, if it works, most useful relationship is the inequality derived by Harker and Kasper. When the only symmetry element is a twofold screw axis, this takes the form:

$$|U_{hkl}|^2 \leq \frac{1}{2} \left\{ 1 + (-1)^k U_{2h,0,2l} \right\}$$

(Ref ²⁴ 29, p. 37).

This can be used to give a sign to a structure factor of the type $h = \text{even}, k = \text{zero}, l = \text{even}$. It will be observed that although signs can be given only to 'even-even' reflections in the $h\ 0\ l$ zone, any one of the general intensities may be involved in the relationship. It is also the case that negative signs may be found if the U_{hkl} on the left-hand side has an odd value of k .

As an initial trial, however, only the $h\ 0\ l$ intensities were examined. No pairs of U's of the type U_h , U_{2h} of sufficient magnitude to make the inequality useful were found.

The 'triple product' relationship was therefore investigated. This states that if $S(h)$ represents the sign of a /

a structure factor of index (h), or a combination of indices represented by (h), then:

$$S(h) S(h') S(h+h') \approx +1$$

where the symbol ' \approx ' means 'probably equals'.

$$\text{e.g. } S(\bar{2}01) \times S(\bar{3}01) \times S(\bar{5}02) \approx +1.$$

A measure of the probability of this sign relation holding was derived by Cochran and Woolfson, who found: (Ref. 24)

$$P_+ = \frac{1}{2} + \frac{1}{2} \tanh \left\{ \left(\frac{\epsilon_3}{\epsilon^3} \right) \left[U_h \times U_{h'} \times U_{h+h'} \right] \right\}$$

where P_+ is the probability and

$$\epsilon_3 = \sum_{j=1}^N n_j^3 ; \quad \epsilon = \sum_{j=1}^N n_j^2$$

n_j having been defined above. The expression ' $\tanh \left\{ \left(\frac{\epsilon_3}{\epsilon^3} \right) \right\}$ '

is approximately constant over reciprocal space, so that P_+ is a function of the product $\left[U_h, U_{h'}, U_{(h+h')} \right]$, and can therefore be plotted against the product.

The method of application was as follows: U's were marked on a drawing of the reciprocal net, and a copy of this made on to a transparent sheet. By moving the origin of the copy to a particular reflection h on the first net, the reflections 'h' and '(h+h\'' were found in pairs superimposed on each other. These were listed systematically, the product

$\left[(U_h)(U_{h'})(U_{h+h'}) \right]$ calculated, P_+ found from a graph of P_+

versus the triple product, and written alongside the list.

Rather arbitrarily, perhaps, it was decided that products with $P_+ \geq 0.90$ would be accepted, and with $P_+ < 0.90$, rejected.

Some 450 triple products, and P_+ for each, were calculated, and from these, three chains of structure factors, the members of each of which probably had the same sign, were

deduced. This was partly made possible by the assumption that all OOL reflections were positive, this being very likely if all the medium atoms lay on $z = 0$. The actual sign for each chain was unknown, and they were given the letters a, b, and c. Eight sets of sign permutation were therefore possible. Only seven of these were calculated as the permutation 'all signs positive' would have given a large peak of electron density at the origin, and the Harker section clearly showed that no atom lay exactly on a screw axis. Consequently, the seven permutations were calculated on Deuce, and the electron density maps plotted.

While no definite conclusions could be drawn from these at the time, comparison with the finally calculated structure factors showed that about 85% of the triple product relationships accepted as being "90% probable" were in fact correct. The three chains amounted in all to 55 reflections, and the most nearly correct permutation had 45 of the 55 signs correct. There are 200 h o L reflections, and a Fourier map derived from 55 of the strongest reflections, 45 correctly and 10 incorrectly signed, might not be expected to give much indication of the correct structure. However, the corresponding electron density map is given (Fig. 5d), along with the final structure.

It will be seen that the regions of highest electron density are very close to the positions of atoms K_2 and P, although K_1 is only poorly represented. Since it was mainly the heavier atoms which were being sought, even these could have been used to find the remaining atoms by a superposition method. Extension of the sign relationships to make use of the general

h k L /

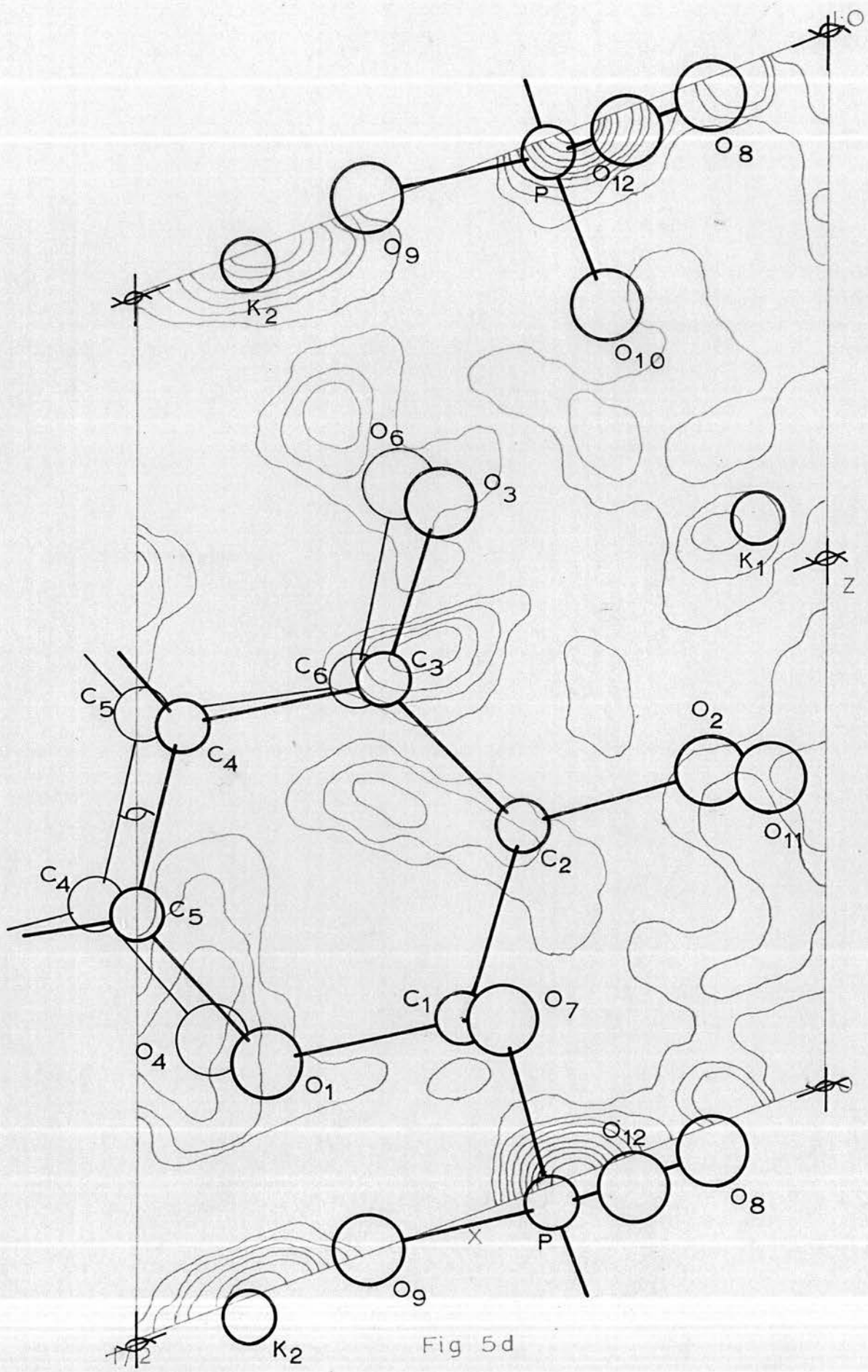


Fig 5d

h k l reflections was under consideration, but was not proceeded with as the crystalline Ammonium salt of Glucose-1-Phosphoric Acid became available at about the same time.

Direct methods, then, even used only to the extent of the simple relationships which could be applied with a desk calculating machine, and to one zone of intensities, did give a crude picture of some of the main features of the structure. In view of this, direct methods, using all the available data, and not merely one layer, might usefully be applied to sugar phosphate structures of about the same complexity as Glucose-1-Phosphate, as a routine procedure, since the rules for their application are quite explicit and require no information except that which is normally available, that is, reflected intensities, space group, and the chemical composition.

The experimental difficulties are twofold. Firstly, since relationships may be established between reflections in quite different regions of reciprocal space, the scaling of all reflections must be fairly accurate. Any anisotropic factor, such as absorption by an irregularly shaped crystal, can make this difficult. Secondly, the most intense reflections are very valuable in establishing inequality relationships or increasing the probability, P_+ , of a triple product being positive. They are also the reflections most subject to extinction, particularly if they are at low angles. The 'chain reaction' nature of sign-determination means that all may depend on accurately evaluating two or three intense reflections, and if one or more of these is affected by extinction, then the process is made that much more difficult, or even impossible.

Conversely /

Conversely, of course, the existence and correct measurement of a few large reflections can lead to a correct structure with comparatively little effort.

Isomorphous Replacement.

While work was proceeding on direct methods, enquiry was made to B.D.H. Ltd. concerning the possible preparation of Ammonium Glucose-1-Phosphate on a small commercial scale. This was successfully prepared and supplied in a very pure state. The material was re-crystallised by the same methods used for the potassium salt, and crystals of a size suitable for X-Ray photography selected. It was notable that excellent crystals were easily grown, with no tendency toward formation of a syrup. Presumably this was possible because of the high purity of the material. Weissenberg photographs of the $h\ 0\ L$, $h\ 1\ L$, $h\ 2\ L$, $h\ 3\ L$, $h\ k\ 0$, $h\ k\ 1$, $h\ k\ 2$, $h\ k\ 3$, and $0\ k\ L$ layers were obtained using a spherical crystal. Calculation of the cell dimensions showed that these were similar to the dimensions of the Potassium salt, being:

$$a = 10.42 \text{ \AA}$$

$$b = 9.09 \text{ \AA}$$

$$c = 7.55 \text{ \AA}$$

$$\beta = 110^{\circ}37'$$

The only systematic absences were among the $0\ k\ 0$ reflections, in the case $k = (2n + 1)$, indicating the space group $P2_1$. At a later stage, photographs were taken with a large crystal and a rather long exposure, in order to detect the weakest reflections, and these showed faint $0\ k\ 0$ reflections at $k = (2n + 1)$. These cannot, so far, be explained.

Density measurements agreed with the value calculated on the basis of two molecules of Ammonium Glucose-1-Phosphate Dihydrate per unit cell, and an analysis for Nitrogen content gave /

gave a percentage corresponding to the formula for a di-hydrate. The preceding evidence suggested that the ammonium and potassium salts were chemically analogous and crystallised isomorphously. The absorption co-efficient was calculated to be 24 cm^{-1} .

Both sharpened and unsharpened Patterson projections down the a, b, and c axes were calculated. Examination of the a- and c- axis were not very instructive, probably because of the amount of overlapping. The b-axis projection possessed a very noticeable peak, marked H_1 on the overlay (Fig. 6) which fell almost exactly on a large peak on the Harker section of the three dimensional Patterson function of the Potassium salt. This was taken to be the Phosphorus-Phosphorus peak. A number of structures based on this assumption were postulated, but none of these were satisfactory, and it was decided that the data should be used in a somewhat different manner. It can be shown (Ref. 22) that if two isomorphous structures are available, say 'm' and 'n', then after putting the F^2 's on the correct relative scale, a Fourier summation with the co-efficients $(F_m^2 - F_n^2)_{hkl}$ gives a vector map similar to the simple Patterson function, but with all vectors other than those from the replaceable atoms to the remaining atoms and to each other removed, this being called the 'difference Patterson'. The relative weights of the peaks, in this case, would be as follows: if Cn = cation, P = Phosphorus, O = Oxygen, C = Carbon then:

$$\text{Cn} \longrightarrow \text{Cn} = (18^2 - 7^2) = 312$$

$$\text{Cn} \longrightarrow \text{P} = (18 - 7) \times 15 = 165$$

$$\text{Cn} \longrightarrow \text{O} = (18 - 7) \times 8 = 88$$

$$\text{Cn} \longrightarrow \text{C} = (18 - 7) \times 6 = 66$$

It //

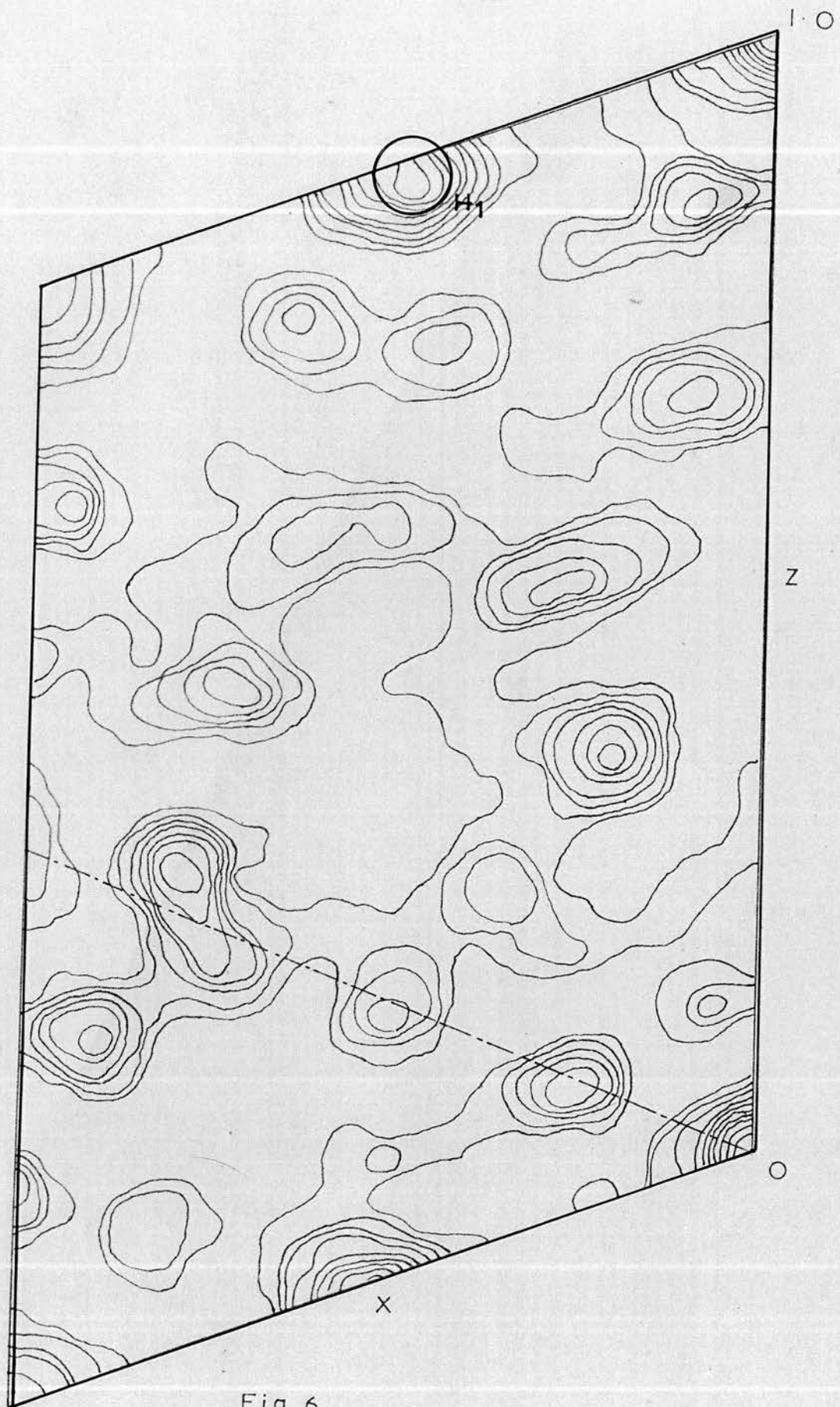


Fig 6

1/2

It is seen that these are more or less in the ratio 4: 2: 1, with C_n C_n as the heaviest vectors. These would, surely, be easily recognised, and the cation positions immediately ascertained. Concurrently with these calculations, the general $h k l$ intensities were being measured, with the help of Mr. P. Rodgers. In fact, the 'difference Patterson' consisted of what appeared to be a random collection of peaks, with no obvious interpretation at all.

Since the $h l L$ intensities had already been estimated, a sharpened, generalised Patterson function was computed as a prelude to the full three-dimensional calculation. The

The three-dimensional Patterson function is :

$$P(U, V, W) = \sum_h \sum_k \sum_l |F_{hkl}|^2 \exp 2\pi i(hU + kV + lW) \quad (1)$$

If we define the generalised projection as :

$$P_K(U, W) = b \int_0^b P(U, V, W) \exp(-2\pi iKV) dV \quad (2)$$

and substitute $P(U, V, W)$ from (1), noting that :

$$\int_0^b \exp 2\pi i(k-K)V dV = 1 \text{ if } k=K \\ = 0 \text{ if } k \neq K$$

then P_K reduces to : $1/A \sum_h \sum_l |F_{hkl}|^2 \exp 2\pi i(hU + lW)$

which with a twofold axis parallel to 'b' becomes :

$$1/A \sum_h \sum_l |F_{hkl}|^2 \cos 2\pi(hU + lW)$$

When $K = 0$, this results in the ordinary projection. When $K=1$, however, we obtain a generalised projection which consists of peaks in the same positions as the $h0l$ projection, but modulated by $\exp(-2\pi iV)$. The Patterson is symmetric, so that the sine term vanishes, leaving the peaks modulated by $\cos 2\pi V$.

$Y = 1$. If we make the simplifying assumption that the Patterson function consists of a set of discrete points, then we can see that the first layer 'generalised' Patterson will be exactly like the projection, except that each point will be multiplied by $\cos 2\pi Y$, where Y is the co-ordinate of that point in vector

space.

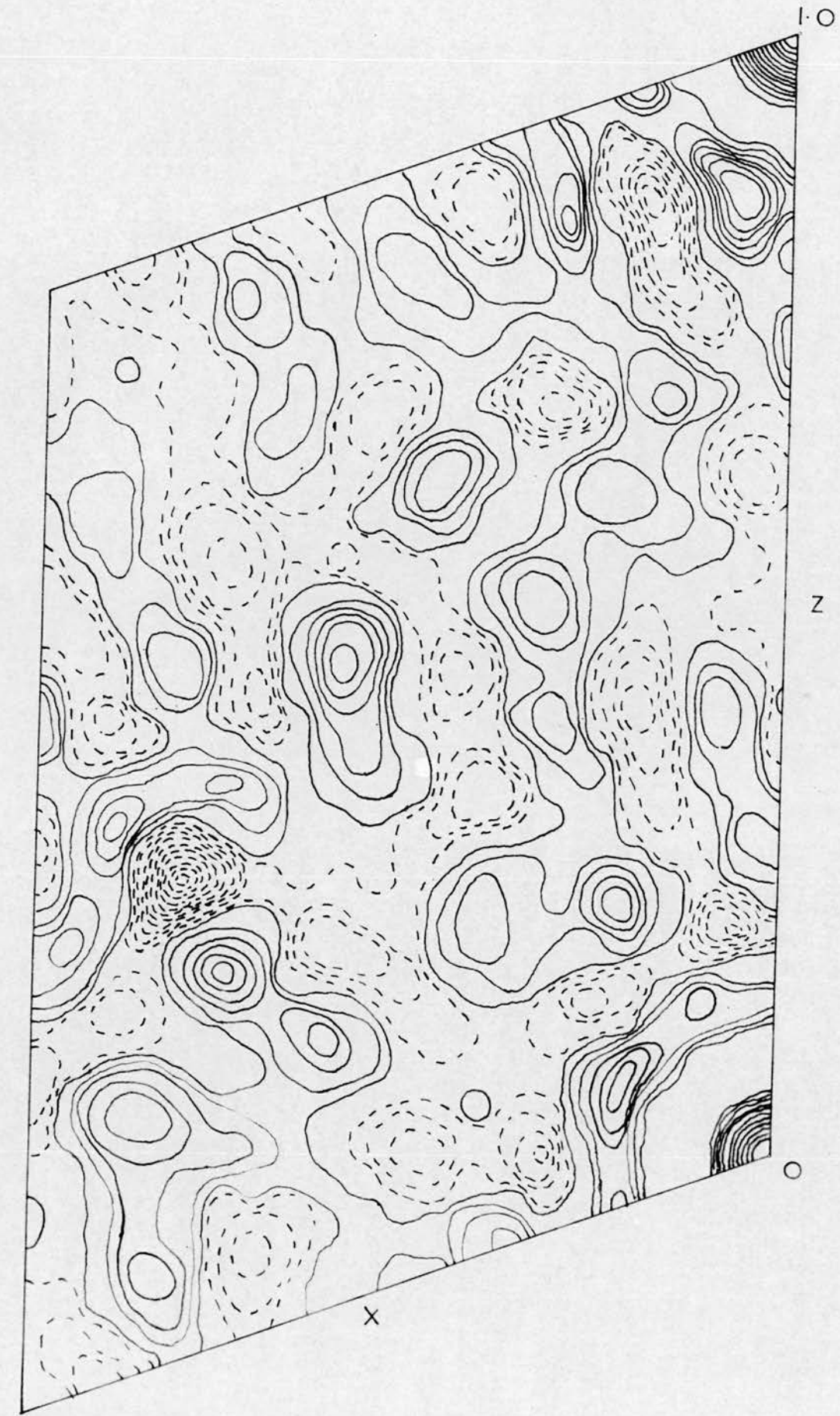
In the Glucose-1-Phosphate structure, it was already well established that the atoms lay mainly in planes with a spacing of $\frac{1}{2}b$. Hence the vector peaks would occur mainly at $Y = 0$, $Y = \frac{1}{4}$ and $Y = \frac{3}{4}$. This meant that the peaks in the generalised Patterson would be multiplied as follows.

At $Y = 0$; by + 1

$Y = \frac{1}{4}$; by 0

$Y = \frac{3}{4}$; by -1 (Harker section).

It may be noted that in the generalised Patterson, negative regions are significant. When drawn out (Fig. 7) it showed only one deeply negative region, and this was not coincident with the peak H_1 on the projection. Hence H_1 could not lie on the Harker section and could not be the P - P vector. This negative peak, however, did lie almost exactly on the short cell diagonal (dotted line, Fig. 6). The peaks around the origin, which should be the other main significant feature of the generalised Patterson (peaks at $Y = \frac{1}{4}$ are multiplied by Zero) also indicated an orientation of the pyranose ring which had the 1.5 Å side of the hexagon lying along the diagonal, whereas the Potassium salt had it aligned with the a-axis. It appeared from this evidence that the h 0 l and h l l photographs had been indexed so that the diagonal of the cell chosen for the Potassium derivative had, quite fortuitously, been used as the a-axis of the Ammonium derivative. The reason for the failure of the difference - Patterson was now obvious, as non-corresponding unit cells were being compared. This could have been repeated, with the corrected indices, but since the general h k l F^2 's had mostly /



1/2

Fig 7

mostly been measured, the three dimensional Patterson function was computed.

In order to emphasise the Phosphorus - Phosphorus and Phosphorus - other atom vectors as much as possible, the sharpening function $(1 + 8 \sin^3 \theta)$, mentioned above, was applied twice over to the data, giving a multiplying ratio of 81:1 in the F^2 's between $\sin \theta = 0$ and $\sin \theta = 1$. This degree of sharpening is quite considerable, and two unsharpened sections were calculated in order to check for possible undesirable effects. A further problem was that, in order to save time, most of the intensities above $L = 3$ had not been measured, and this would lead to elongation of all the peaks in the Z - direction. In fact, neither the high sharpening nor the cutting off of the data along one axis had any ill effect. Sharpening improved the resolution very satisfactorily and the sharp termination of data affected only the largest peaks, and then to a minor extent.

The expected vector pattern round the origin, showing the pyranose ring orientation, appeared very clearly. Also present were four peaks each at about 1.5 \AA from the origin, and arranged in a manner corresponding to that expected from a tetrahedral group. These were the vectors from the Phosphorus atom to its covalently bonded Oxygens, and were sufficient to determine the orientation of the phosphate unit. Finally, in the sphere around the origin, there was a peak at about $X = 0$, $Y = 1.4 \text{ \AA}$, $Z = 0$. Since the pyranose ring plane was known to be perpendicular to the b-axis, and only the 1-position is axial, this /

this vector peak presumably included the C_1 - ester oxygen (O_7) link, and perhaps also the vector from C_6 to O_6 , this being the only other possibility. The sections in the region of $Y = \frac{1}{4}b$ showed, as expected, the Phosphorus - Glucose ring vectors. A fair approximation to the shape and orientation of most of the anion was therefore now known, and it remained to fix the positions of the cations, of this anion, and of the water molecules.

A direct comparison of the sections $Y = 0, 1, 2, 3, 17, 18, 19,$ and $20 - \frac{1}{40}b$ showed clearly the reason for lack of success in finding the cations. The centre-centre vector must be present on both Patterson maps. It follows from this that a vector present on one but not the other cannot be the centre-centre vector. The maxima on the Ammonium salt map which had diminished relative to their counterparts on the Potassium salt map must correspond to vectors with the cation at one end. Examination of the sections listed above showed that on the Potassium derivative vector map, certain of the larger peaks must be $K^+ - K^+$ and $K^+ - P$ vectors. In the region of $Y = \frac{1}{8}b$, these included a number of peaks which had formerly been considered as possible centre-centre vectors. Indeed, they included all the larger peaks, the centre-centre peak being very much smaller than had been supposed. The Harker section of the Ammonium salt gave the position of the Phosphorus atom, and with the nature of the larger vectors now definitely established, a heavy atom arrangement was devised which accounted convincingly for them. Structure factors in the $h\ 0\ l$ layer were calculated for /

for the three atoms K_1 , K_2 and P alone, and the agreement obtained with the observed magnitudes was satisfactory, in view of the fact that these atoms did not contain an outstandingly large proportion of the electron content of the cell (about 28%), and the somewhat approximate location of them.

Since the positions of the atoms in the pyranose ring and the oxygen atoms of the phosphate group relative to the Phosphorus atom were known, location of this atom was sufficient to place the others. It had already been suggested that the peak H_1 on the b-axis Patterson projection of the Ammonium salt was, in fact, the centre-centre peak, and the position of the Glucose ring determined via the Phosphorus atoms also lead to a centre-centre peak at H_1 . The co-ordinates of the freely rotating O_6 and also of the water molecules, O_{11} and O_{12} , were as yet unknown. In an attempt to obtain these, a three dimensional minimum function (Ref. 22, p. 239) was prepared graphically from the Patterson map of the Ammonium derivative. The origin was displaced to coincide with the Phosphorus-Phosphorus peak, as an "image" of the structure relative to the heaviest atom present must possess the greatest weight. In displacing the origin to the Harker section, it had to be borne in mind that the resulting minimum function would have the space group $P2_1/m$, leading to a solution plus its mirror image. It was presumed that the ambiguity could be resolved for the required atoms, if found, on packing considerations. As for the glucose residue, this was assumed to be the D-form, although this could not be proved by the X-Ray methods available.

The /

The minimum function did give a rough outline of the sugar ring in the position deduced previously, giving further confirmation of its correctness, but the remaining atoms were not found. An 'idealized' structure was then made up, consisting of the K^+ and P positions, as far as they were known, and a perfect cyclohexane ring, set in the correct orientation and with its centre on the position indicated by the assumed centre-centre peak. Certain ring addenda, O_2 , O_3 , O_4 and C_6 were placed on the assumption that C_1 must be the nearest ring atom to the Phosphorus, and that the Glucose ring was similar to that of pure Glucose. The positions of the Oxygen atoms of the Phosphate group were deduced from the P - O vectors around the origin. Structure factors of the type $h\ 0\ l$ with $\sin \theta \leq 0.85$ were calculated for these atomic positions, and agreement was good enough to proceed with an electron density difference map of the b-axis projection. This showed that some small changes in atomic parameters were required, and also three distinct peaks which were taken to be O_6 , O_{11} , and O_{12} . The insertion of oxygen atoms on these positions, and recalculation of structure factors, this time up to $\sin \theta = 1.0$, gave an R - value ($R = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|}$) of about 0.42. Further difference maps and small shifts reduced this to 0.29, unobserved reflections being inserted as $F_{ob} = 0$.

At this stage, a model was built of the structure, as it was so far known. O_6 and the water molecules, O_{11} and O_{12} , were tentatively allocated Y-co-ordinates on spatial considerations, i.e. that O_6 should be about 1.4 - 1.5 Å from C_6 , and that /

that all should be at least 2.6 - 2.9 Å from non-bonded neighbours. Structure factors for the h k 0 zone were computed, and after three difference-Fourier cycles, R was approximately 0.25.

It was obvious, however, that the amount of atomic overlap in all three projections was considerable, and although refinement by projections alone could have been continued there appeared to be some advantage in three-dimensional refinement. Consequently, some 1,400 general h k L structure factors were computed for the co-ordinates deduced from projections. The R-value was 0.35. A three-dimensional electron density map was computed, and the maximum for each atomic position found by interpolation using the method of A.D. Booth (Ref. 25). The co-ordinates obtained were only very slightly different, although the heights of the maxima were rather variable, indicating possibly incorrectly located atomic positions. The fact that an F(obs) Fourier strongly tends to return atomic positions similar to those put in is well known, the main indication of wrong position being reduced electron density. One of the water molecules, O₁₂, was particularly in doubt. The h k 0 reflections were re-calculated for the atoms other than O₁₂, and a difference map computed. The latter indicated a position for O₁₂ whose Y-co-ordinate was increased by some 0.45 Å. This substantial shift was made, and the h k 0 reflections re-calculated, with a marked improvement in agreement. A further ($\rho_o - \rho_c$) map showed that a slight tilt of the entire sugar ring was required. This was very small /

small, too small to be apparent on the Patterson function, but it meant that the line $C_2 - C_5$ no longer lay exactly perpendicular to the screw axis, which helped to explain the fact that in the Patterson function, the centre-centre peak was much more diffuse than had been anticipated. Not clearly indicated by the Fourier maps was a shift of one of the phosphate oxygens, O_{10} ; this was deduced from an examination of the angles in the phosphate group. A shift of about 0.3 \AA was required. Making all these alterations gave an $(h k 0)$ disagreement index of about 0.16, with unobserved reflections set equal to $\frac{1}{\sqrt{2}}$ X minimum observed.

Concurrently with refinement of the c-axis projection, the b-axis projection was also being refined, though none of the atomic shifts required was as large as those outlined above. Calculation of the three-dimensional structure factors was carried out, using the revised co-ordinates, and gave a disagreement factor of 0.27. In order to confirm the arrangement of the phosphate group, structure factors were calculated without the phosphate-oxygens, and an electron-density difference map computed. This only led to a very slight change in the positions, but indicated clearly that the factor which allows for thermal vibration of the Phosphorus atom must be very small, i.e. the vibration of the Phosphorus atom is of small amplitude. On re-calculating the general structure factors, with the improvements in positional and vibrational parameters deduced from this map, a slight improvement in agreement was obtained, and with an R-value of 0.24, refinement by the method of least squares was started.

Least Squares.

In the refinement of a crystal structure, the aim is essentially to vary the parameters of the atoms in the unit cell until the calculated structure factors give the best agreement possible with the observed values, under the prevailing experimental conditions. In doing this, an attempt is made to minimise the sum $\sum ||F_o| - |F_c||$, or some similar function. The observed F's, however, are subject to a range of errors, as are all experimental measurements, and the refinement process therefore consists of getting the best fit of calculated and observed data, the latter being subject to error.

The principle of least squares states that the most probably correct set of parameters is obtained when the sum of the squares of the deviations between calculated and observed values is minimised, after allowing for the reliability, or 'weight' of each observation (Ref. 34) i.e. by minimising $R' = \sum W(|F_o| - |F_c|)^2$, where W is a weighting function for each reflection. If the trial structure is sufficiently close to the correct one, we can, as Hughes first pointed out, set up a number of linear equations, thus:

$$\sum_i^n \frac{\partial F}{\partial P_i} \cdot \delta P_i = F_o - F_c = \delta F$$

There is one such equation for each F, and $P_1, P_2, P_3 \dots P_n$ are the variable parameters which must be optimised.

For /

This is complicated by two main factors. Firstly, the coefficients vary with each change of parameter, which means that the solution can only be arrived at by successive approximations. Secondly, the number of equations is very large. In the present case of a twenty-atom structure with three positional and six vibrational parameters per atom, plus one overall scale factor, there are 181 equations to be solved per cycle of refinement. This can be circumvented by making use of the fact that the principal diagonal consists of the sums of squares, which are large and positive, whereas the off-diagonal terms are the sums of products, which are as likely to be negative as positive and therefore may be quite small, on average. Refinement of the structure can therefore proceed on the diagonal terms only, since they will tend to outweigh the others. When this approximation is made, the parameter shifts become equal to:

$$\Delta P_i = \frac{\sum w \left(\frac{\partial F_i}{\partial P_i} \right) \Delta F_i}{\sum w \left(\frac{\partial F_i}{\partial P_i} \right)^2}$$

Since it is no longer necessary to compute the off-diagonal terms in the first place, this approximation leads to a reduction /

reduction also in the effort required to set up the normal equations, and not only in solving them.

Unfortunately, although off-diagonal terms are much smaller on average, a few may be of comparable magnitude to the diagonal terms, which leads in turn to a slower convergence on the correct solution. This, however, is unavoidable in the absence of equipment capable of solving such large sets of simultaneous equations.

The three-dimensional least-squares refinement of Glucose-1-Phosphate was carried out on the D.E.U.C.E. Computer, using the programme of J.S. Rollett (Ref. 28). The weighting system was as follows:

$$|F_o| \leq 32 \quad ; \quad \sqrt{w} = |F_o|/32$$

$$|F_o| > 32 \quad ; \quad \sqrt{w} = 32/|F_o|$$

The reason for choosing this weighting is that the root-mean square 'F' is about 24 - 30, and this weighting system would give greatest weight to reflections of about average, or slightly greater than average, intensity. The influence of the weighting system on convergence is hard to determine, but it seemed reasonable that reflections in this intensity region should be the most accurate, and hence of greatest weight. The decrease in R per cycle was very small, and after five cycles using the 1,350 general reflections it was 0.21. A further cycle suggested that this was about the lowest value for the data available, and that a plot of R versus parameter would have a rather flat minimum. It was apparent from the fact that the discrepancies were /

were more marked at lower $\sin \theta$ values that the probable cause of error was mainly anisotropic absorption, which could only be partially compensated by the facility for anisotropic refinement of the thermal vibration parameters. In addition, for a few strong reflections, extinction was almost certainly present e.g.

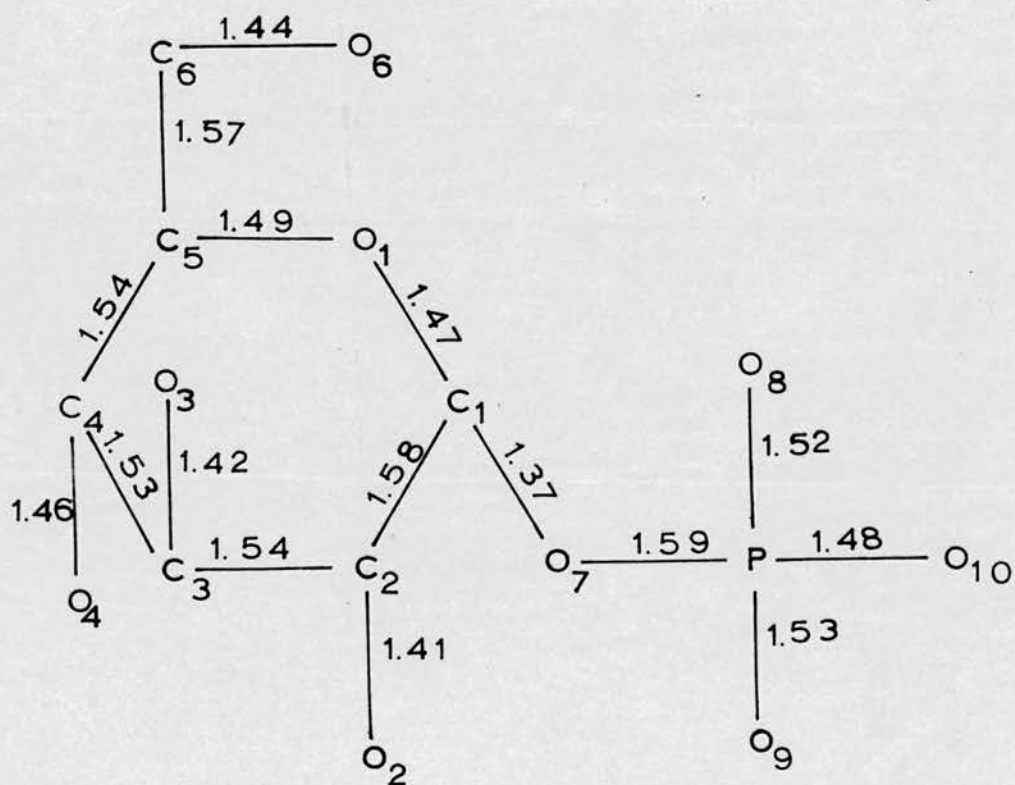
$\sin^2 \theta$	h	k	L	F_o	F_c
0.075	$\bar{3}$	2	1	62.0	90.5
0.039	$\bar{1}$	2	1	37.0	62.0
0.039	0	2	1	49.5	97.7

Two cycles of refinement were therefore carried out on more limited data, about 960 reflections, which had been measured using an almost cylindrical crystal and corrected for absorption by assuming an exactly cylindrical form. The R-value was then reduced to 18.6%, after scaling the F_{ob} against F_{calc} in ten equal ranges of $\sin^2 \theta$. The parameters obtained are given below, those for thermal vibration being the mean values, as the anisotropic components are more probably related to absorption rather than vibration.

Atom	$\frac{X}{a}$	$\frac{Y}{b}$	$\frac{Z}{c}$	B
K_1	0.948	0.454	0.439	3.1
K_2	0.421	0.002	0.986	3.46
P	0.203	0.510	0.984	1.4
O_1	0.406	0.227	0.221	2.7
O_2	0.087	0.225	0.337	2.9
O_3	0.284	0.195	0.694	3.5
O_4	0.551	0.298	0.735	2.5
O_6	0.684	0.302	0.275	3.6
O_7	0.238	0.419	0.177	2.7
O_8	0.084	0.609	0.980	1.9
O_a	0.332	0.599	0.003	2.1

Atom	X/a	Y/b	Z/c	B
O ₁₀	0.161	0.405	0.823	1.3
O ₁₁	0.056	0.204	0.602	1.0
O ₁₂	0.852	0.430	0.023	2.8
C ₁	0.266	0.271	0.193	2.6
C ₂	0.223	0.103	0.353	2.7
C ₃	0.323	0.252	0.514	2.7
C ₄	0.471	0.209	0.572	2.8
C ₅	0.502	0.281	0.405	2.8
C ₆	0.656	0.248	0.438	2.8

The bond lengths are given diagrammatically below :



Using the approximate formula of Cruickshank (Ref. 30) for standard deviations of co-ordinates:

$$\sigma(x) = \frac{R}{S} \left(\frac{N}{4P} \right)^{\frac{1}{2}}$$

where:

R = Residual

\bar{S} = r.m.s. value of $(2 \sin \theta / \lambda)$ for the planes considered.

P = difference between number of reflections observed and number of variable parameters.

N = number of atoms of the type being examined required to give the same amount of scattering at \bar{S} as that of the entire asymmetric unit.

$$\text{i.e. } \sum_i f_i^2 = N f_x^2$$

one obtains, for each chemical type:

$$\text{K} \quad \sigma = 0.008 \text{ \AA}$$

$$\text{P} \quad \sigma = 0.009 \text{ \AA}$$

$$\text{O} \quad \sigma = 0.024 \text{ \AA}$$

$$\text{C} \quad \sigma = 0.034 \text{ \AA}$$

Using the expression for bond-length deviations:

$$\sigma^2(l) = [\sigma^2(x_1) + \sigma^2(x_2)] \cos^2 \alpha + [\sigma^2(y_1) + \sigma^2(y_2)] \cos^2 \beta + [\sigma^2(z_1) + \sigma^2(z_2)] \cos^2 \gamma$$

where l = bond length

and

$$\cos \alpha = \left(\frac{x_1 - x_2}{l} \right), \quad \cos \beta = \left(\frac{y_1 - y_2}{l} \right), \quad \cos \gamma = \left(\frac{z_1 - z_2}{l} \right)$$

errors are obtained for each type of bond which do not vary from

significantly from the following averages:

Type.	Probable error.	
C - C	\pm 0.05	\AA
C - O	\pm 0.04	\AA
P - O	\pm 0.03	\AA

Intramolecular /

Intramolecular bond angles are as follows, to the nearest degree:

P - O ₇ - C ₁	124°	O ₂ - C ₂ - C ₃	110°
O ₁₀ - P - O ₇	109°	C ₂ - C ₃ - C ₄	111°
O ₈ - P - O ₉	112°	C ₂ - C ₃ - O ₃	109°
O ₈ - P - O ₁₀	111°	O ₃ - C ₃ - C ₄	111°
O ₈ - P - O ₇	105°	C ₃ - C ₄ - C ₅	106°
O ₁₀ - P - O ₉	114°	C ₃ - C ₄ - O ₄	103°
O ₉ - P - O ₇	105°	O ₄ - C ₄ - C ₅	103°
O ₇ - C ₁ - C ₂	113°	C ₄ - C ₅ - O ₁	111°
O ₇ - C ₁ - O ₁	116°	C ₅ - O ₁ - C ₁	111°
O ₁ - C ₁ - C ₂	108°	C ₄ - C ₅ - C ₆	107°
C ₁ - C ₂ - O ₂	113°	C ₆ - C ₅ - O ₁	114°
C ₁ - C ₂ - C ₃	106°	C ₅ - C ₆ - O ₆	108°

Discussion of the Structure.

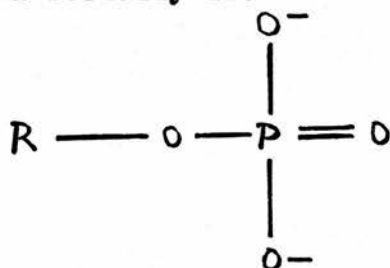
In the molecular structure, the average C - C bond length is 1.55 Å, the average C - O length is 1.44 Å, and average P - O = 1.53 Å. The bond C₁ - O₇ is shorter than average by almost twice the C - O standard deviation, and may therefore be significant. Both McDonald with α - Glucose (Ref. 34) and McGeachin with the similar α - Rhamnose (Ref. 36) observed a shortening of the bond from C₁ to its hydroxyl group, though in the case of Glucose, this has been queried by Killean, Ferrier and Young (Ref. 35). A. Miller (Ref. 37) found no such shortening of the axial C - OH bond in Myo-Inositol. No shortening of the O - ring carbon bond occurs in Cytidine - 3' - Phosphate, and any such bond shortening must therefore be associated /

associated with the presence of a ring oxygen atom adjacent to point of attachment and possibly only in the case of an axial substituent.

The bond $P - O_7$ is twice the $(P - O)$ standard deviation longer than average, which may also be significant. This lengthening of the $P -$ bridge oxygen bond has been found in the phosphate esters mentioned earlier and Cruickshank (Ref. 38) discusses theoretical reasons for the extension of bonds of this type. $P - O_{10}$ is just less than twice the S.D. shorter than average. A similar shortening of one of the free $P - O$ bonds was also observed in Cytidine - 3' - Phosphate⁽⁸⁾ and in Dibenzyl-⁽³⁹⁾ Phosphoric acid,⁽⁹⁾ though not in A.M.P., Calcium Thymidylate,⁽¹¹⁾ or 2 - Aminoethanol Phosphate.⁽¹⁰⁾ The $P - O - R$ angle in Glucose - 1 - Phosphate is 124° , which does not differ greatly from the 121° of Cytidine-3'-Phosphate and Dibenzyl-Phosphoric acid. In the other structures studied so far, the corresponding angle is between 115° and 119° . It is seen then, that α -Glucose-1-Phosphate, Cytidine-3'-Phosphate, and Dibenzyl Phosphoric acid all possess one free $P - O$ bond which is shorter than the others, and a rather wide $P - O - R$ angle. There is no obvious reason for this; the feature which these molecules have in common, but which is absent in the other phosphate esters examined, is that the phosphate group is esterified directly to a ring system, and not on to a side chain. Since only six structures are being compared, however, it is quite possible that this correlation could /

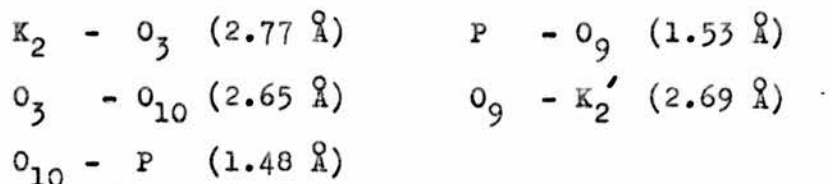
could arise by chance. The rings to which the phosphate group is attached are, for one thing, all different; one furanose, one pyranose, and one aromatic.

Discussion of such variations ultimately must depend on the accuracy of the structure, or, at least, the trust one places in the calculated standard deviations, and on the establishment of a 'normal' value for the quantity being considered. The normal values of the C - C and C - O bond lengths have been very well established from the large number of structures containing these bonds which have been independently studied. The situation with respect to the free P - O bonds in organic phosphates is rather different, for while these can be written down formally as:



which would make one expect one short bond, the observed values certainly do not give consistent support to this expectation.

The crystal structure and atomic numbering is given in Appendix (4). It can be pictured as a scaffolding consisting of a chain formed by the following atoms:



where K'_2 is the symmetry related counterpart of K_2 . This chain runs /

runs parallel to the b-axis and can continue indefinitely. The bond P - O₉ is one of the longer free P - O bonds and may be partially charged, providing an electrostatic attraction between O₉ and K₂. The Glucose ring is held in this frame by the links

O₆ - K₂ (2.61 Å)
 O₄ - O₉ (2.63 Å)
 O₃ - O₁₀ (2.65 Å)
 and possibly O₃ - K₂ (2.77 Å).

The other cation K₁, is surrounded by a 'shell' of oxygen atoms at the following distances:

K ₁ - O ₂ = 3.05 Å	K ₁ - O ₁₂ = 3.15 ^{2.94} Å
K ₁ - O ₆ = 2.94 Å	K ₁ - O ₂ ' = 2.78 Å
K ₁ - O ₁₁ = 2.93 Å	K ₁ - O ₁₁ ' = 2.79 Å

(O₂' and O₁₁' are in the unit cell adjacent to that containing K₁).

It is apparent, then, that the units forming the crystal are fairly tightly bonded together, as might be expected from the observed hardness and lack of any easy cleavage. The forces involved appear to be a combination of ionic and 'hydrogen-bond' types, additional evidence for the latter being found in the infra-red absorption spectrum of the solid, ground up in an organic liquid, which shows considerable absorption in the 3000 cm⁻¹ region usually associated with the hydrogen bond.

Methods Employed.

It is noticeable in the descriptions of the structure determinations of the phosphate esters mentioned in the foregoing sections that in all cases but one a useful trial structure was obtained by means of some vector method or methods. The exception is the structure of Barium Ribose-5'-Phosphate, found by means of the 'heavy atom' method. It also seems to be the case that the presence of a medium or heavy cation is more of a hindrance than a help, being large enough to enhance absorption problems considerably without aiding the solution of the structure by very much. Certainly, in work of this class of compound, careful choice should be made of a suitable derivative. This choice, of course, will be limited by the number of derivatives which can be prepared in a crystalline form. The experiences outlined in the appropriate references (8, 9, 10) and in the examination of Ammonium Glucose-1-Phosphate show that if vector superposition methods are employed the necessarily present Phosphorus atom should be quite heavy enough to lead to a good trial structure. This is not because Phosphorus is particularly heavy ($Z = 15$) - in fact, it is far too light to use, say, the direct 'heavy atom' method, but because the P - P vectors are just about heavy enough to be recognizable on a vector map unambiguously, and, even more important, the phosphate must be covalently bound to the sugar residue. This means that in a superposition map, or even in the Patterson itself, although the symmetry is higher than that of the structure, it becomes easier to trace a chain of permissible other-atom positions, each atom being about 1.5 \AA from /

from the next.

A good deal of very useful information can also be got from the volume around the origin of a vector map, though this does not seem to have been utilised except in the work on Glucose-1-Phosphate.

It has already been explained how a light-atom molecule will give rise to a distinct pattern around the origin if it contains a number of parallel vectors capable of reinforcing each other. This is the situation which applies in the case of the pyranose ring, and also in the pyrimidine and purine groups, when present. The usefulness of this area of vector map, however, is not restricted to groups with parallel vectors. Any small unit of known shape, some of whose vectors may be expected to be distinguishable from the background can be orientated from the vector distribution around the origin. This occurs with the P - O vectors of the tetrahedral P - O₄ group.

Location of the actual position of a molecule in the unit cell could be done either by using a superposition map, derived from the P - P vector or vectors, in which case a prior knowledge of the possible orientations of the units involved would be of considerable assistance in detecting the correct structure among the inevitable unwanted 'chance' coincidences, or by means of a centre-centre type of vector if one is possible. In the latter case it is necessary to remember that this will only exist in projection, since the sugars are optically active and cannot crystallise in a centrosymmetric space group. Further to this, atomic groups which are not centro-symmetric /



centrosymmetric may possess centrosymmetric projections. In the case of Glucose-1-Phosphate, the $-PO_4$ group is orientated with one of its $\bar{4}$ axes parallel to the b-axis, leading to a phosphate 'centre-centre' peak located at the same X, Z, co-ordinates as the P - P Harker vector, making this, in projection, a heavier peak even than single K - K or K - P vectors.

For light-atom derivatives of sugar phosphates, sharpening is rather advantageous, though disapproved by at least one authority (Ref. 22, p. 266). The enhancement of P-P and P \rightarrow other atom peaks is considerable, without excessive series termination effects appearing. This is also commented on by Kraut and Jensen⁽⁹⁾ in their work of Adenosine - 5' - Monophosphate. The $|F|^2$'s of Di-Ammonium Glucose-1-Phosphate Dihydrate were subjected to a sharpening ratio of $(1 + 8 \sin^3 \theta)^2$, and both the Glucose ring intramolecular vectors and the phosphate group vectors appeared quite clearly, although they are close to the origin, where series termination errors should be worst. By contrast, the Patterson function of Di-Potassium Glucose-1-Phosphate Dihydrate, both unsharpened and slightly sharpened, gave a relatively poorer, though recognisable, pyranose vector pattern and no identifiable $-PO_4$ pattern. This was despite the fact that it contained more coefficients, about 1350 for the Potassium salt as opposed to about 700 for the Ammonium salt.

The superiority of three dimensional over two-dimensional vector methods is already well known, and the extra labour/

labour of measuring the larger number of reflections required is very little compared to the advantages to be gained. The advent of fast computing equipment over the last few years has made the calculation involved in preparing, say an unsharpened, slightly sharpened, and strongly sharpened version of the same Patterson the least of the problems to be faced in a structure determination.

In the course of refinement, both electron density difference maps and the method of Least-Squares were used. It had been hoped that the latter would give a more rapid refinement, than was possible by Fourier methods, especially in the closing stages when shifts were small, and might be more accurately determined by this method than by examination of an electron density map. In practice, convergence proved to be extremely slow. It is possible that this was caused by the omission of the off-diagonal terms in the normal equations.

Crystallisation of Sugar Phosphates.

The first step in any structure determination is the obtaining of suitable crystals. Glucose-1-Phosphate presented no serious difficulty here. Attempts were also made to crystallise some other phosphates, if possible. Fructose 1 - 6 Diphosphate, which is of considerable importance in photosynthesis, is fairly readily available, and experiments were made with this substance. The commercial Barium, Calcium and Magnesium salts are all amorphous, powder photographs of each of these showing no evidence of crystallinity. The Potassium, Rubidium, Ammonium, Copper, and Zinc salts were all prepared from the Barium salt by reaction in solution with the appropriate inorganic sulphate. The copper and zinc derivatives were not sufficiently soluble to achieve the concentration necessary for satisfactory crystal growth, while the other derivatives were rather hygroscopic, forming glassy solids on dehydration. It is also probable that crystallisation was hindered by excessive impurity in the starting materials, the purity of many biochemicals being rather variable.

Reference was found to the use of Cyclohexylammonium (40) and Cyclohexylamine (41) salts for the purification of certain sugar phosphates. Cyclohexylammonium salts were hardly likely to be suitable for X-ray structure determinations on account of the multiplicity of puckered rings which must necessarily be present. Cyclohexylamine was therefore considered more promising.

The Cyclohexylamine salt of Fructose 1-6-Diphosphate was prepared by passing a solution of the soluble Calcium salt through a cation exchange column in the Hydrogen form and neutralising the free Fructose-1-6 Diphosphoric Acid with Cyclohexylamine /

Cyclohexylamine. The resulting solution was filtered and dried in a vacuum desiccator with Sodium Hydroxide as desiccant. The resulting powder was recognised as consisting of fine needles when observed under the microscope. A powder photograph also showed it to be crystalline. The powder was then redissolved and dried out again more gently, at a lesser degree of vacuum and using anhydrous Calcium Chloride as desiccant. This led to a number of clusters of very fine needles, radiating from various growth points. No single crystal of a size suitable for X-ray photography was obtained, but an oscillation photograph of a bundle of parallel needles showed that the crystals possessed a symmetry axis parallel to the needle axis, with a cell translation in this direction of about 6.0 \AA . The most probable interfering reaction in the method of preparation was hydrolysis of the phosphate on the ion exchange column, which would have led to a mixture of Cyclohexylamine Orthophosphate and Fructose. Fructose, however, crystallises in almost cubic crystals, with unit cell dimensions of 8.06 \AA , 10.06 \AA and 9.12 \AA (Reference 42); while Cyclohexylamine Orthophosphate, which was prepared as a check, crystallised in flat, mica-like plates. The Cyclohexylamine salt of Fructose-6-Monophosphate has also been prepared, though time has not permitted a full examination of methods of crystallising it.

Looking both at the experiments on Fructose 1 - 6 Diphosphate and the description in Reference 41 to the crystallisation of Deoxyribose - 1 - Phosphate, it seems that organic /

organic bases can be usefully employed to form crystalline derivatives of sugar phosphates which can not be crystallised with a metallic cation. While this will complicate the structure by the increased number of atoms present, the range of possible derivatives, one or more of which may be crystalline, is greatly increased.

A C K N O W L E D G E M E N T S .

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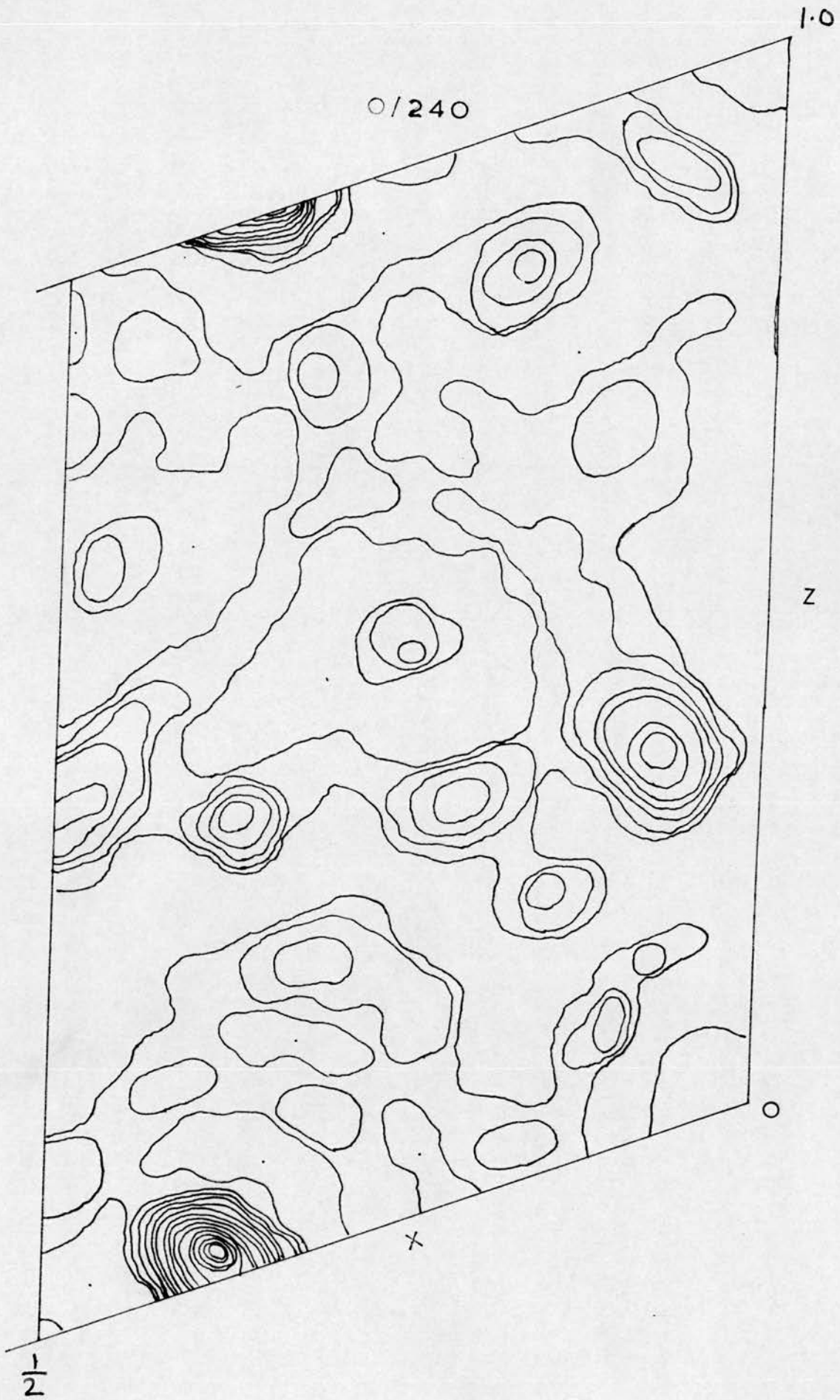
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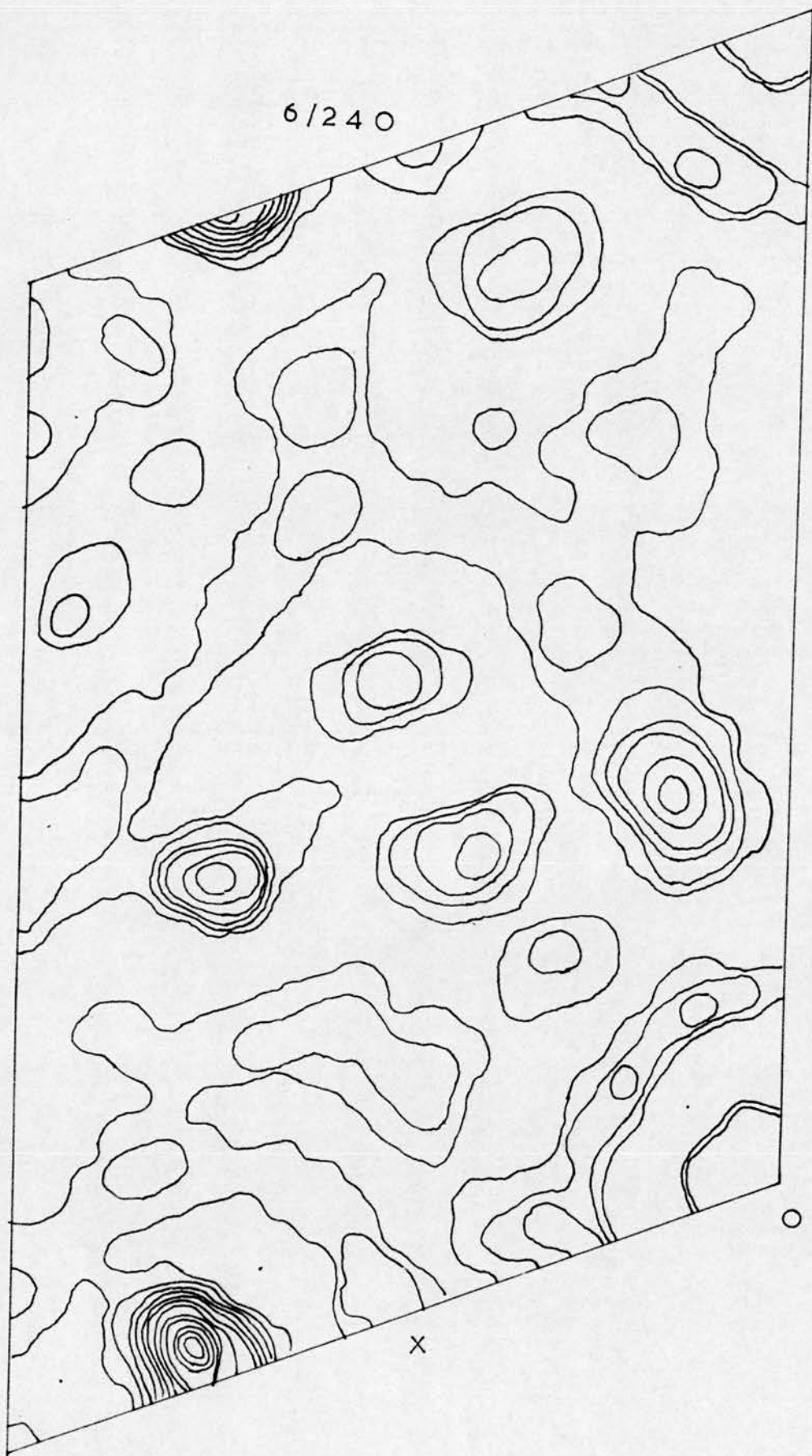
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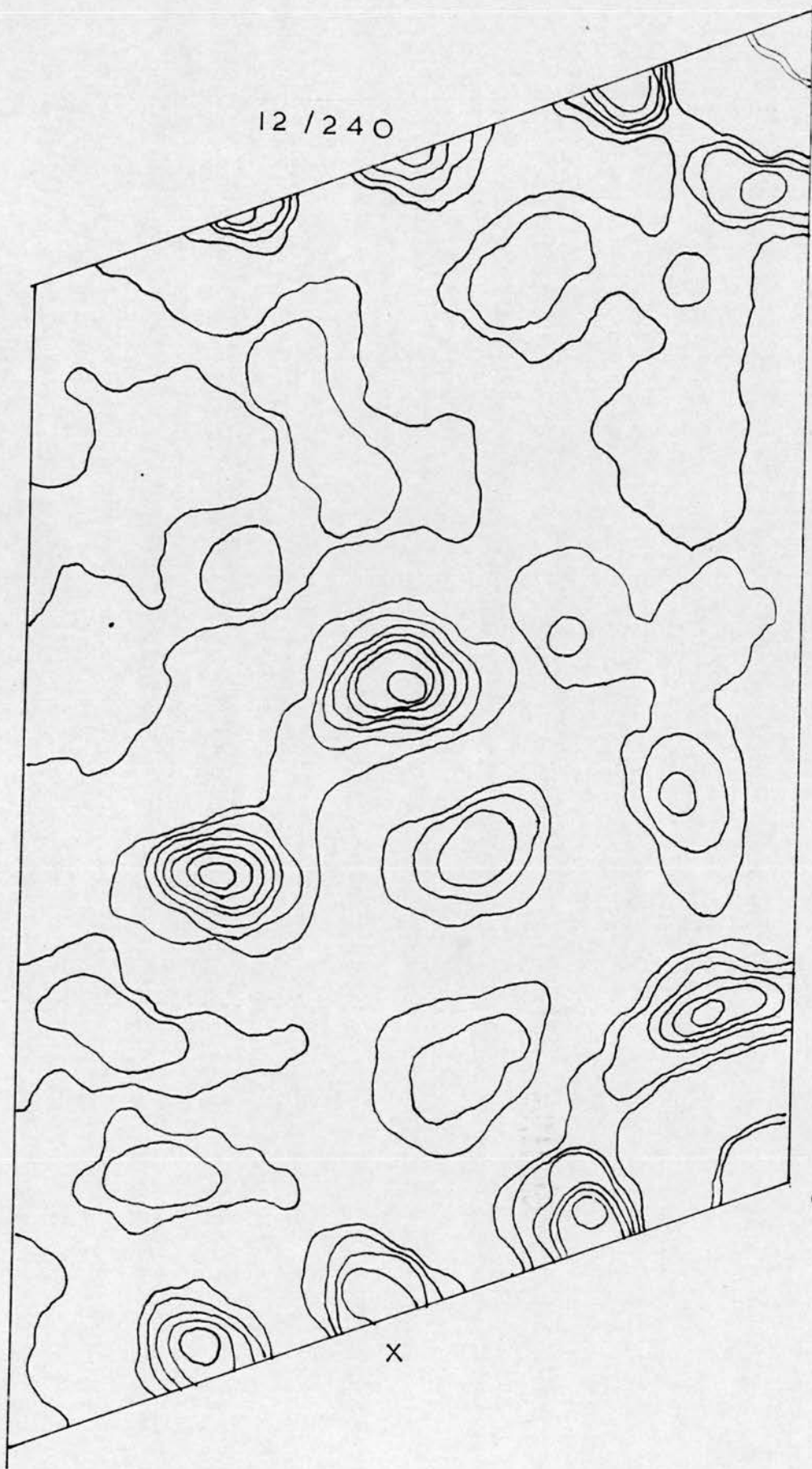
APPENDIX (1)

Sections of the three-dimensional Patterson function of Dipotassium Glucose-1-Phosphate Dihydrate, taken at right angles to the b -axis. The appropriate y -level is indicated at the top of each section.





12 / 240

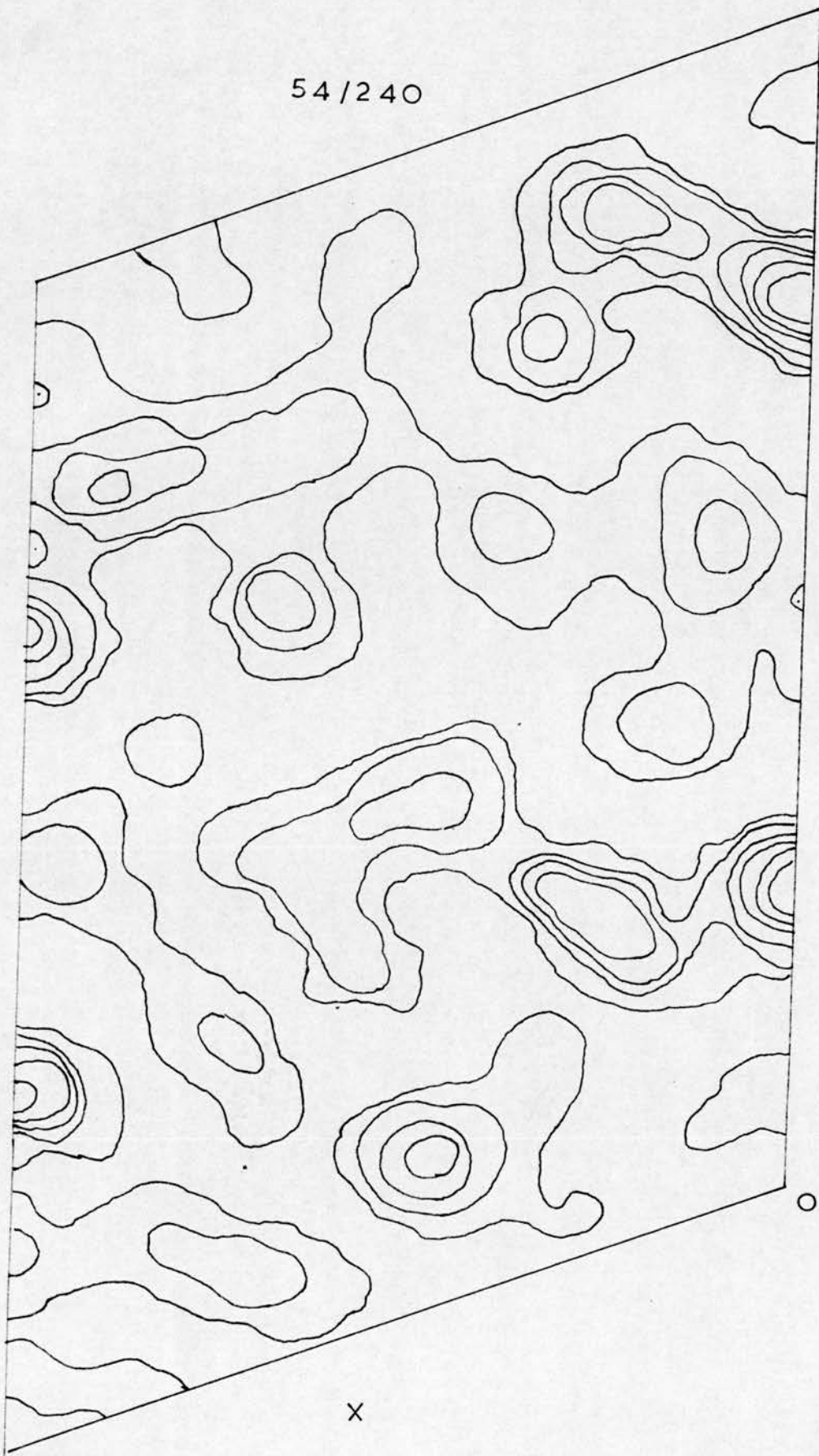


Z

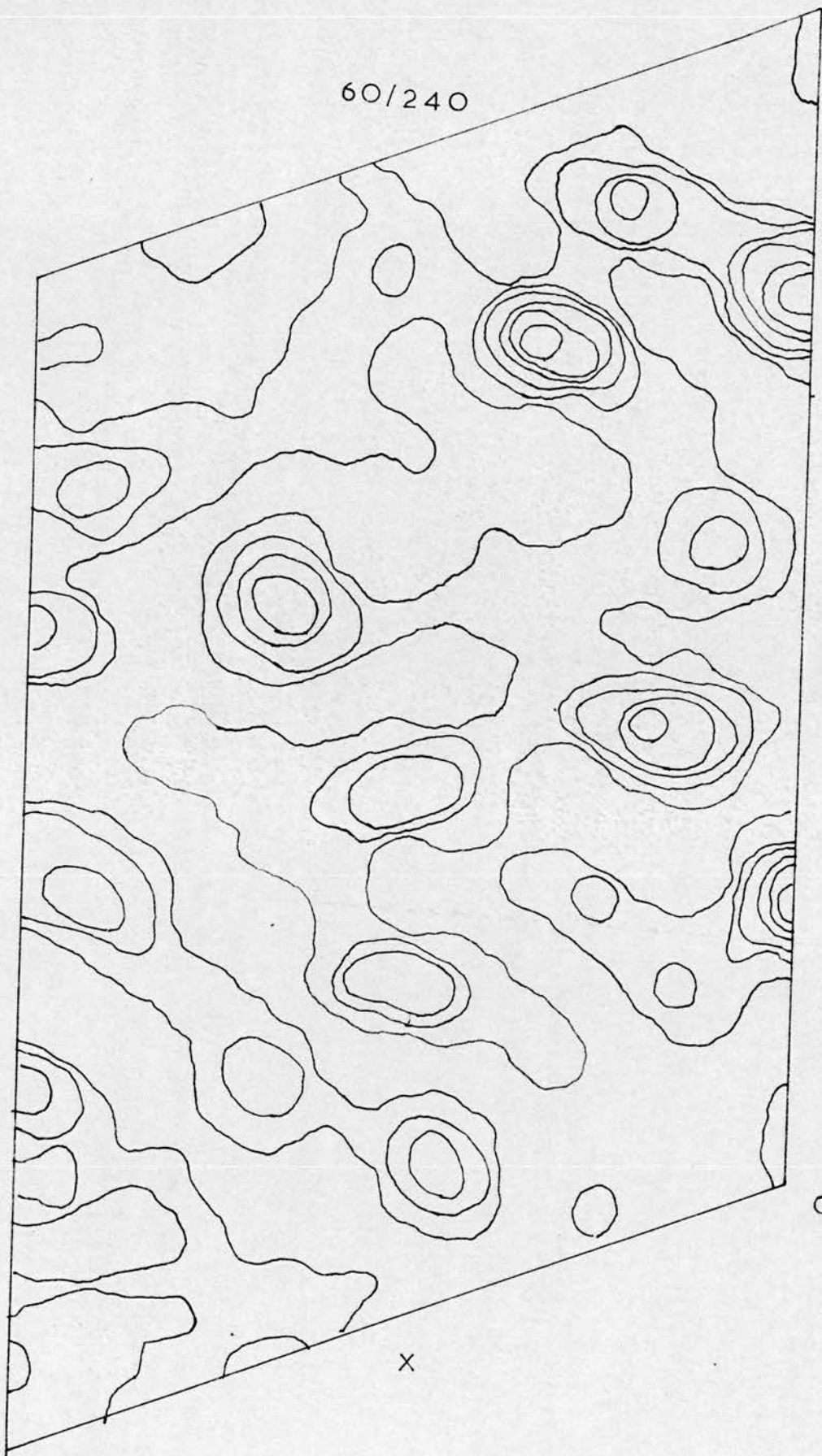
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60/240

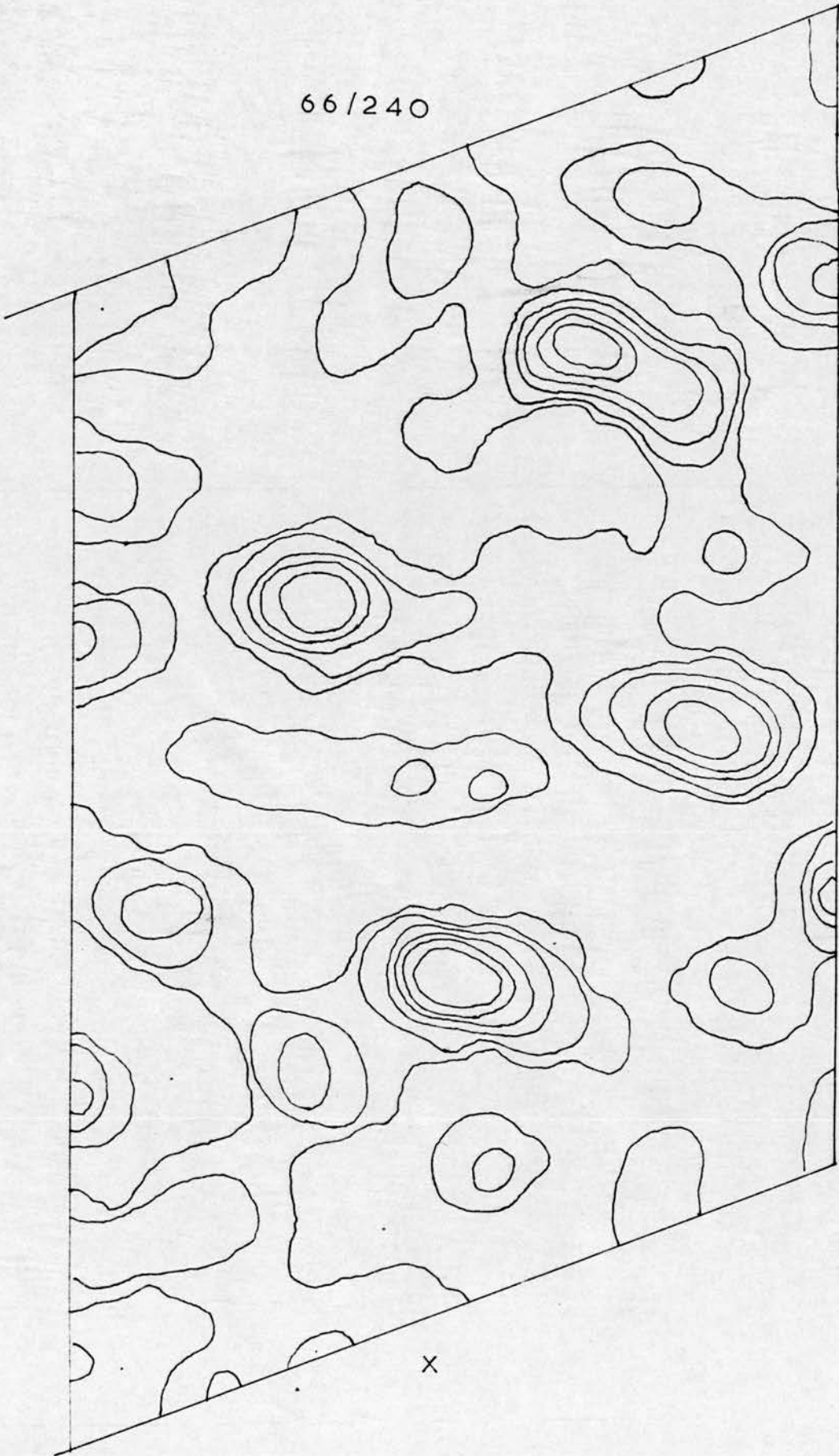


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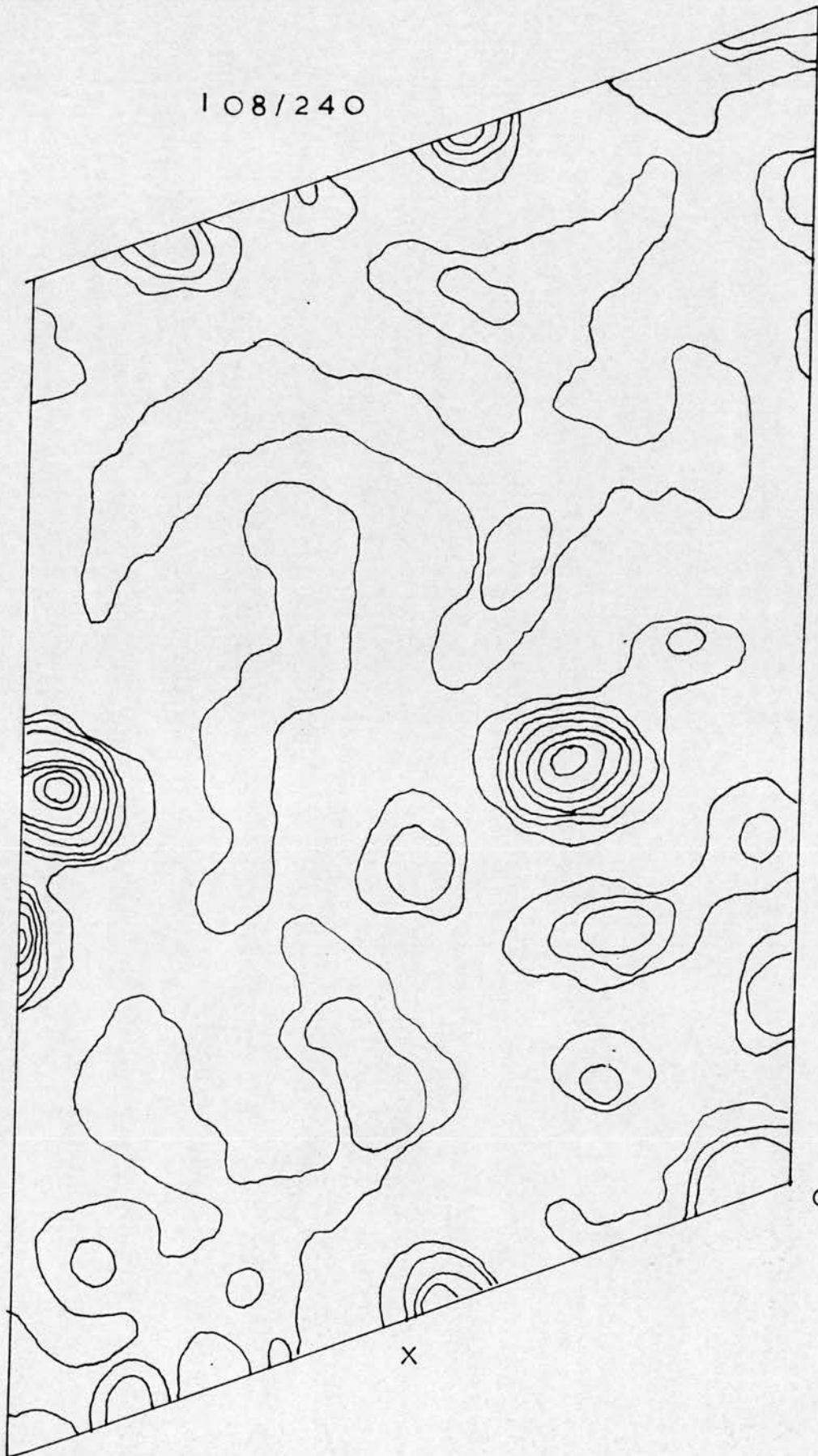


Z

X

O

108/240

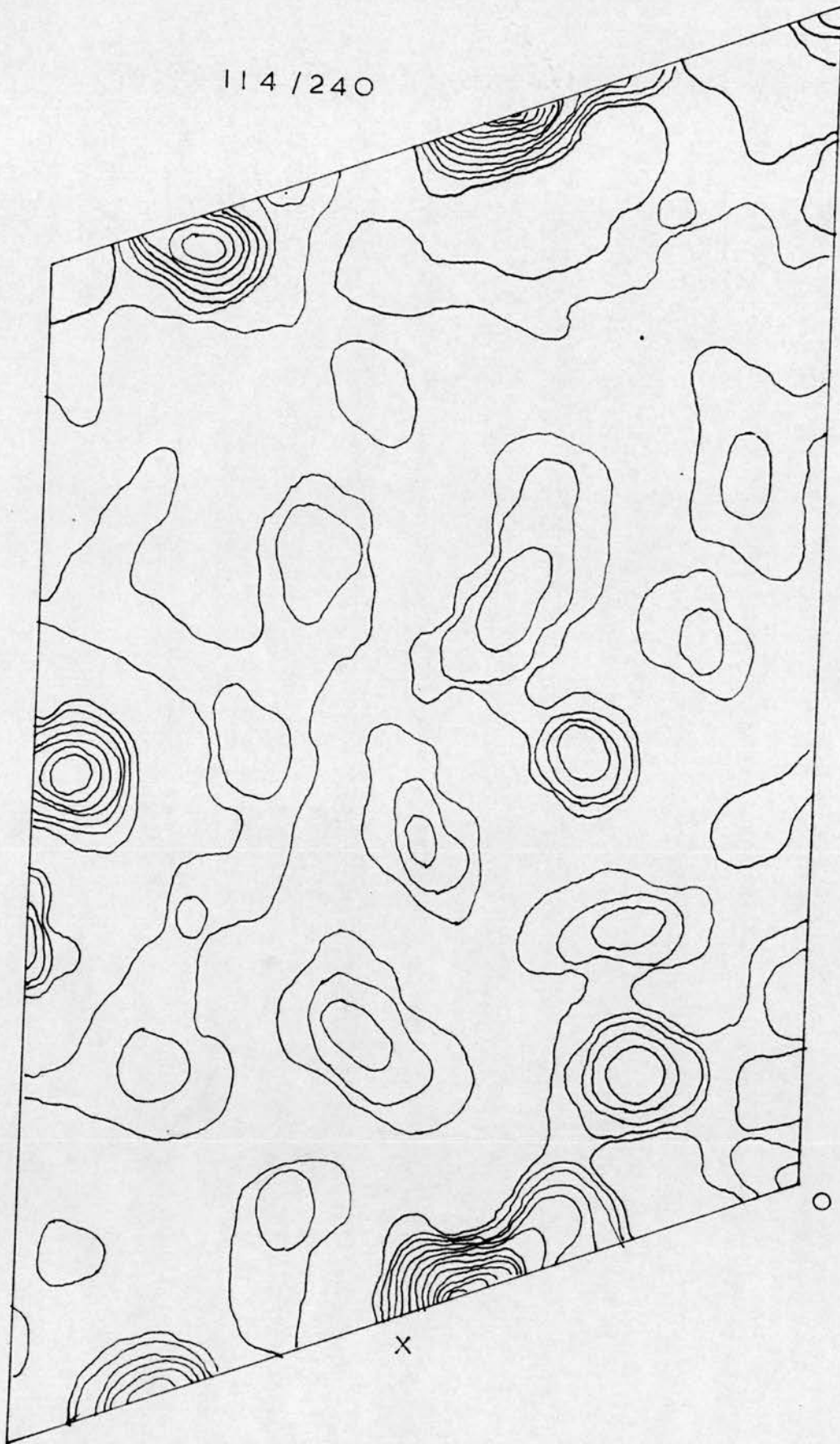


Z

X

o

114/240

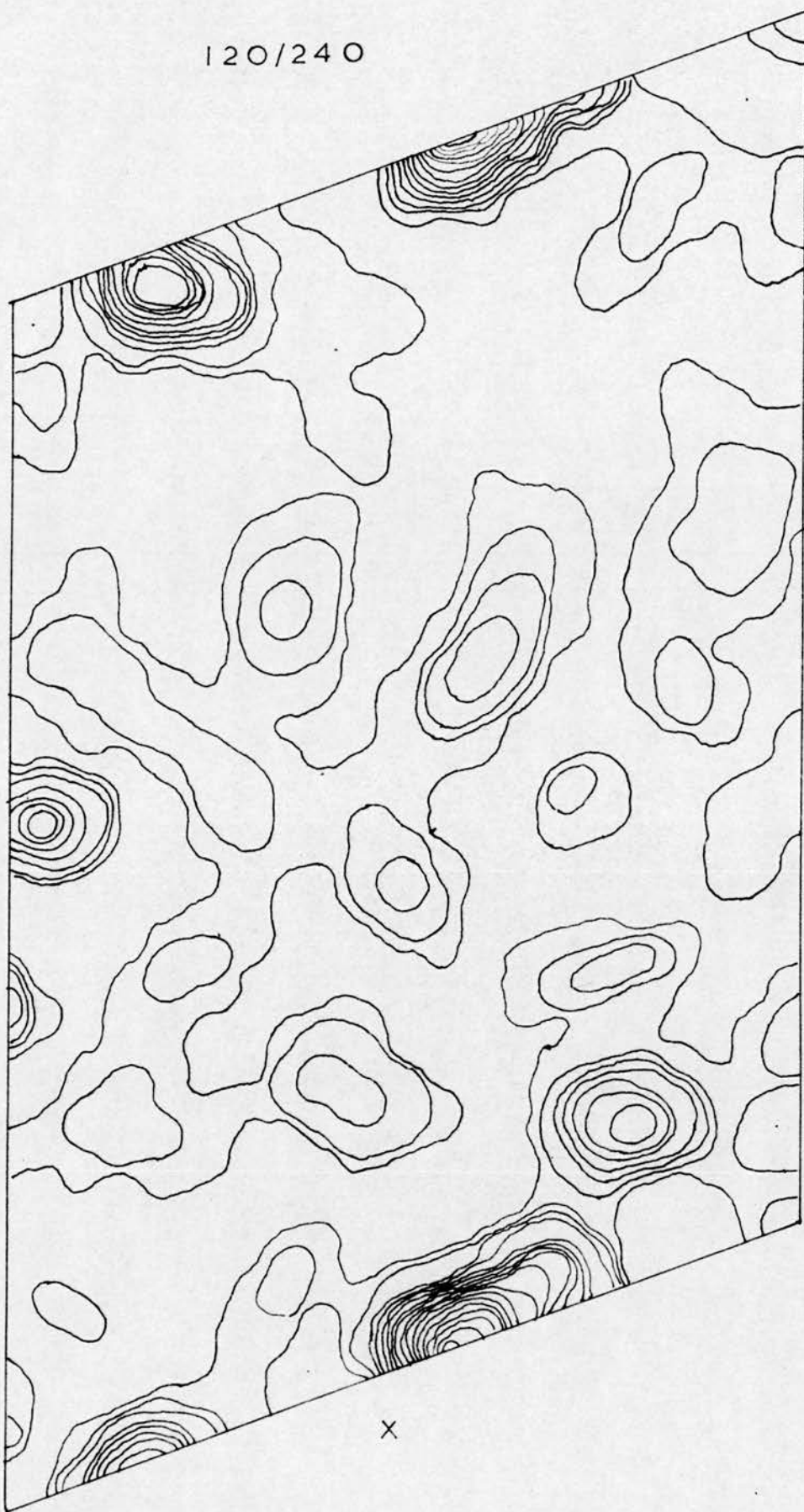


z

x

o

120/240



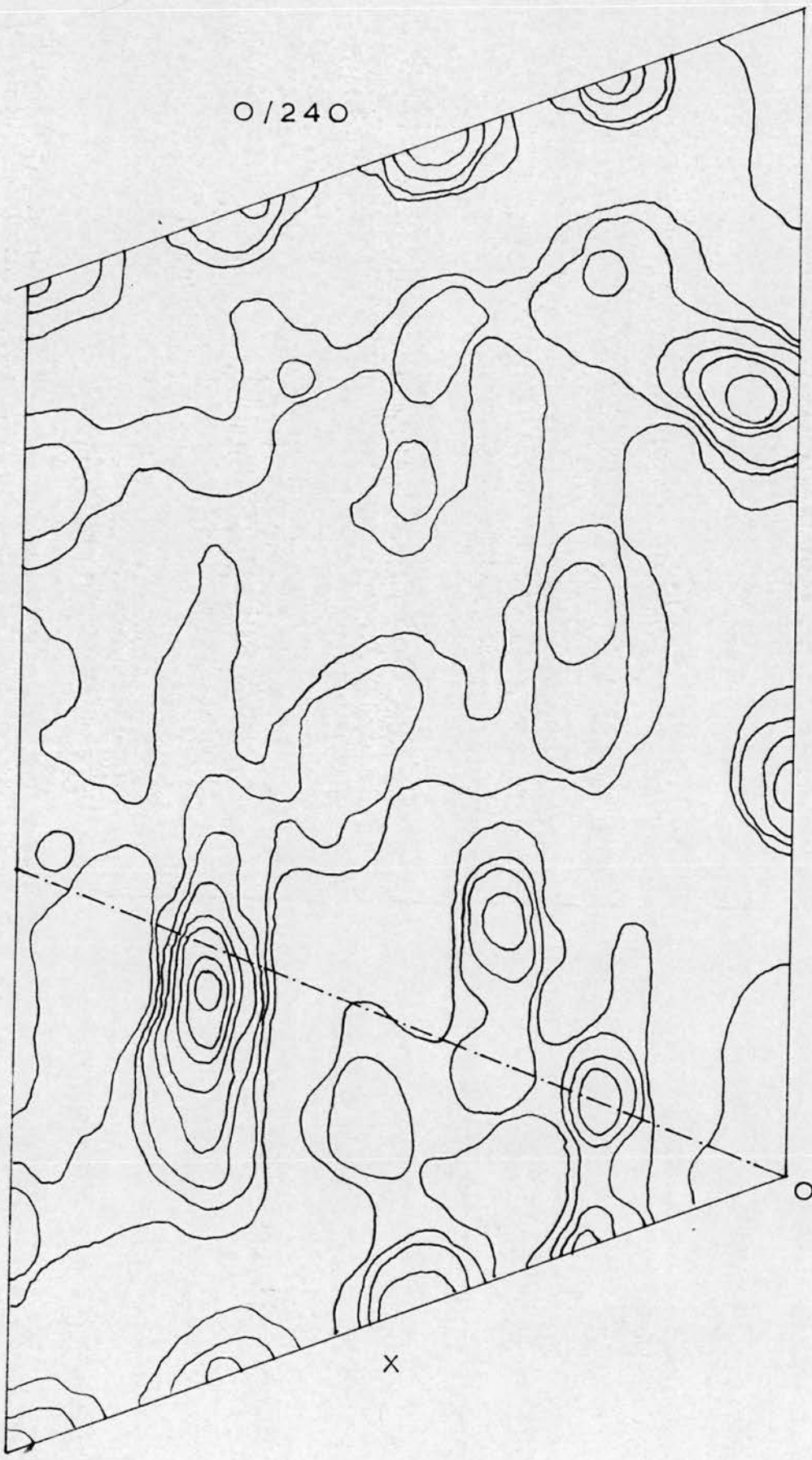
Z

X

O

APPENDIX (2)

Sections of the Patterson function of Di-ammonium
Glucose-1-Phosphate Dihydrate, set out as in Appendix (1).

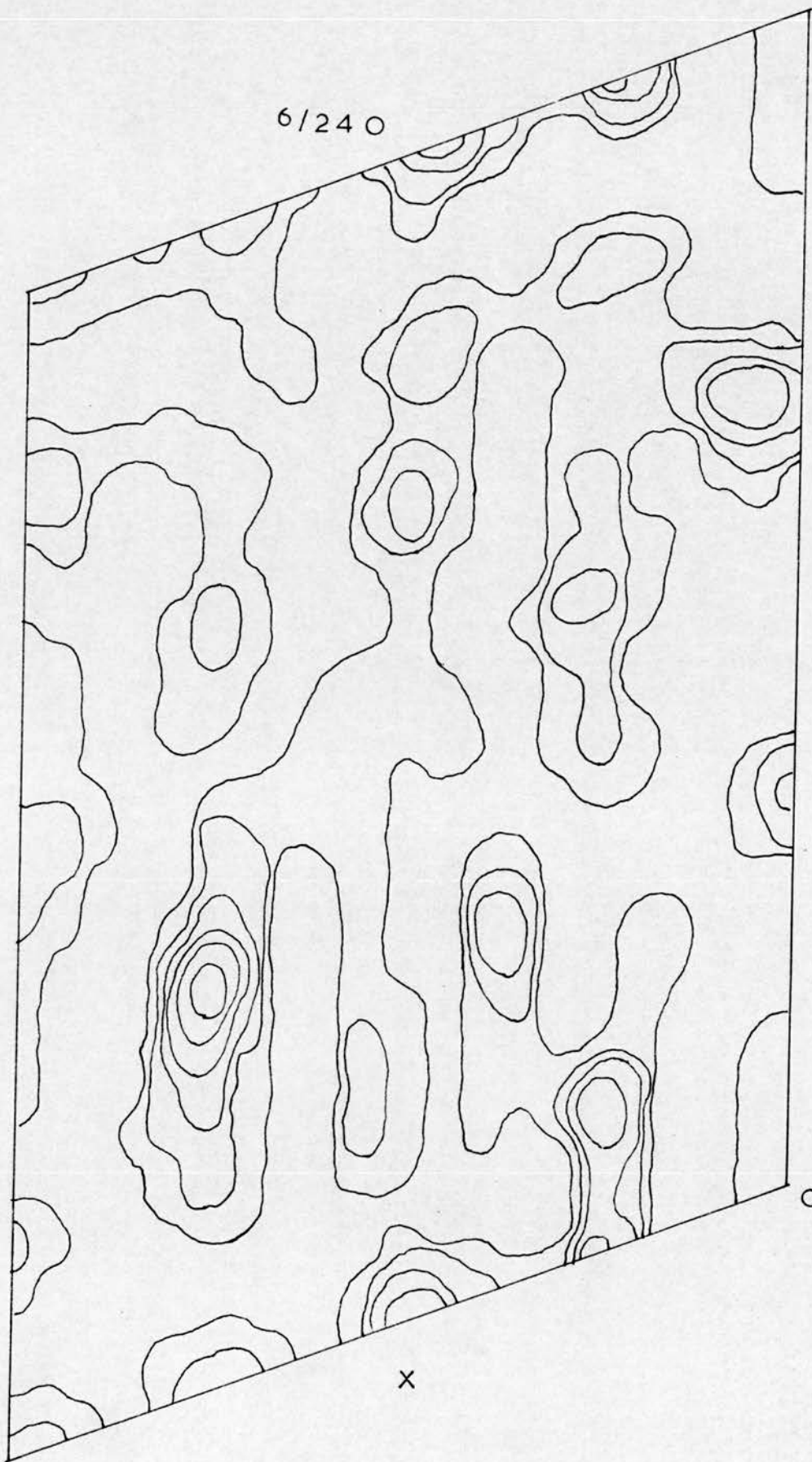


O/240

Z

X

6/24 0

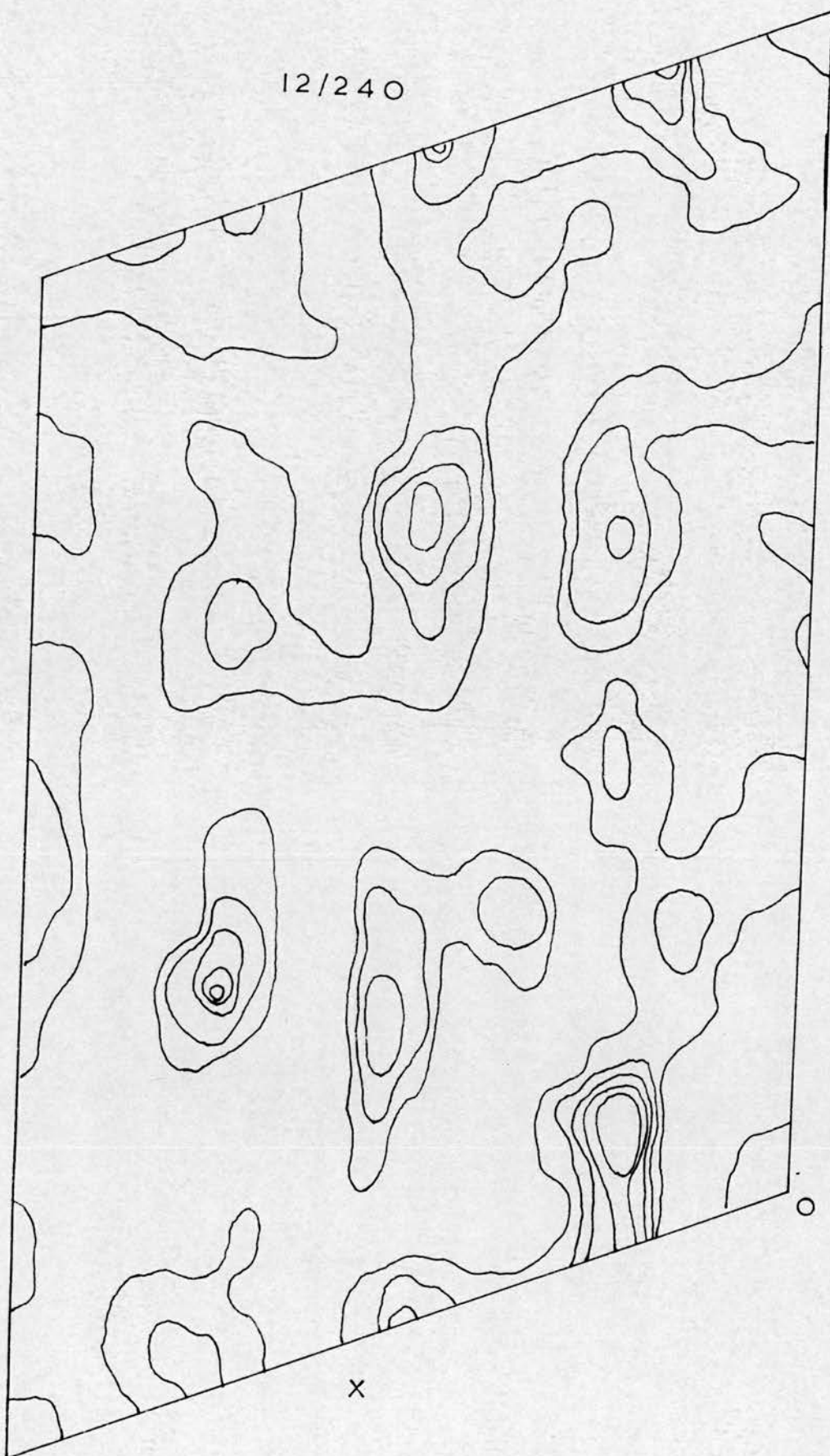


z

x

0

12/240

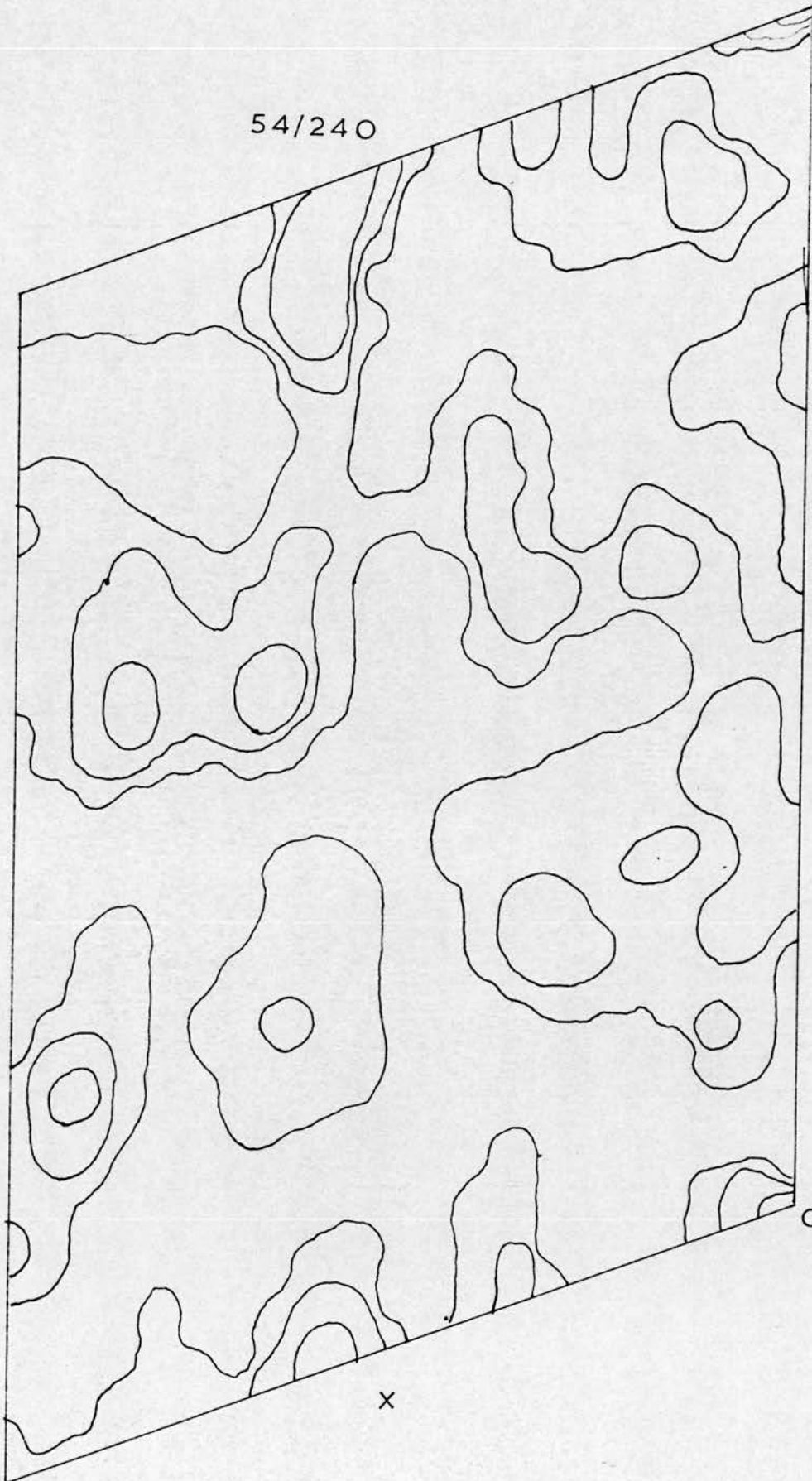


z

x

o

54/240

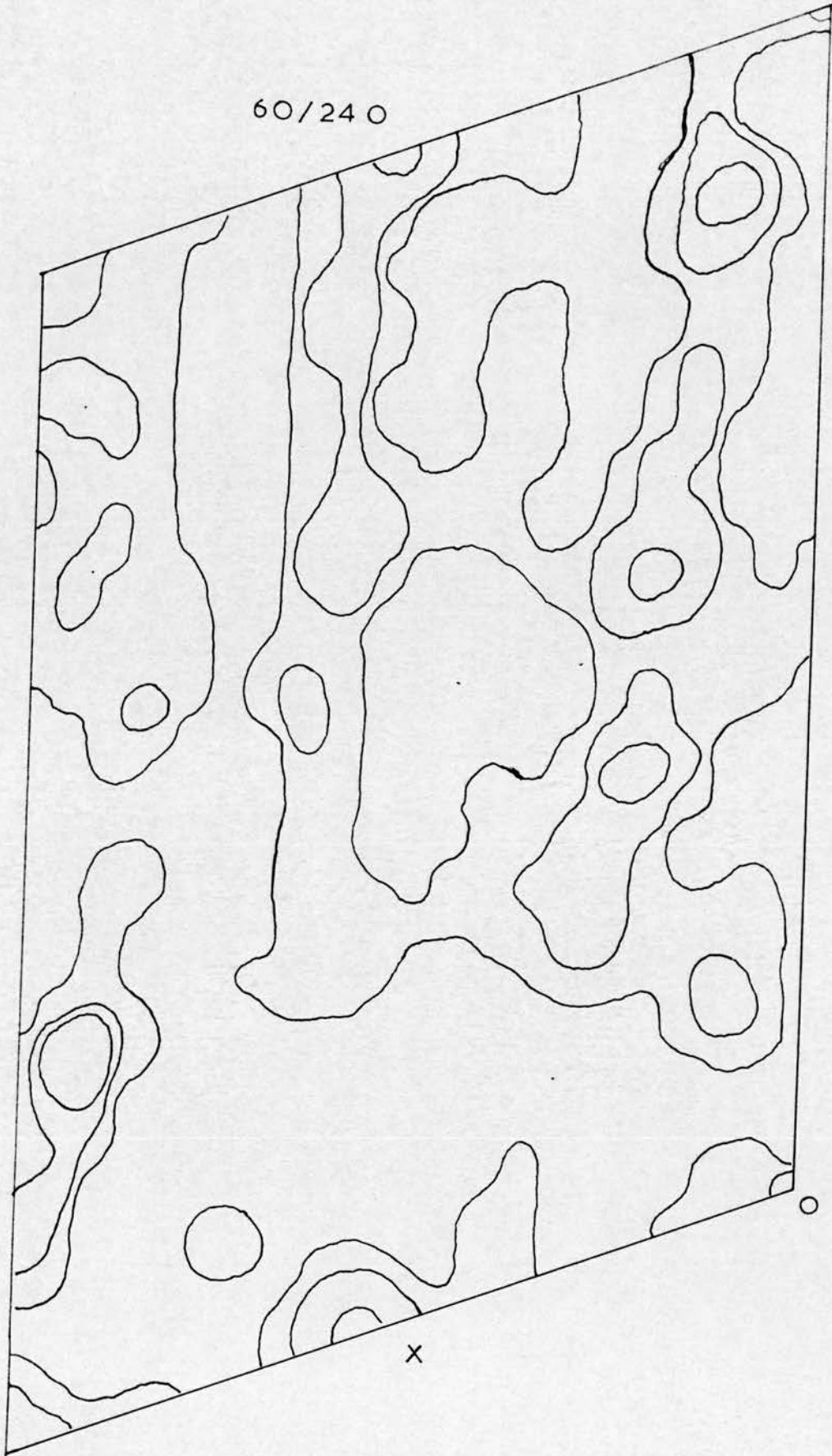


z

x



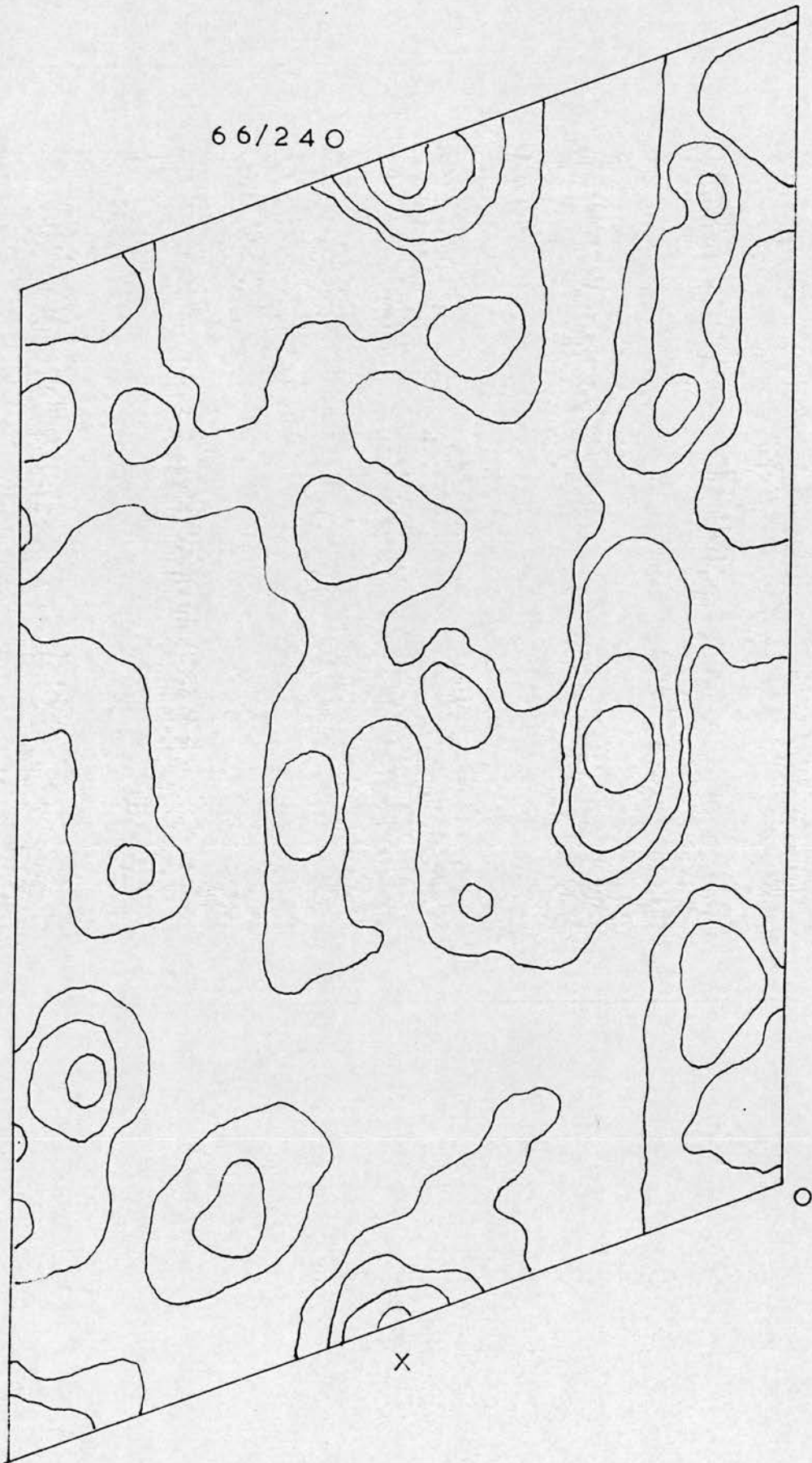
60/240



z

x

66/240

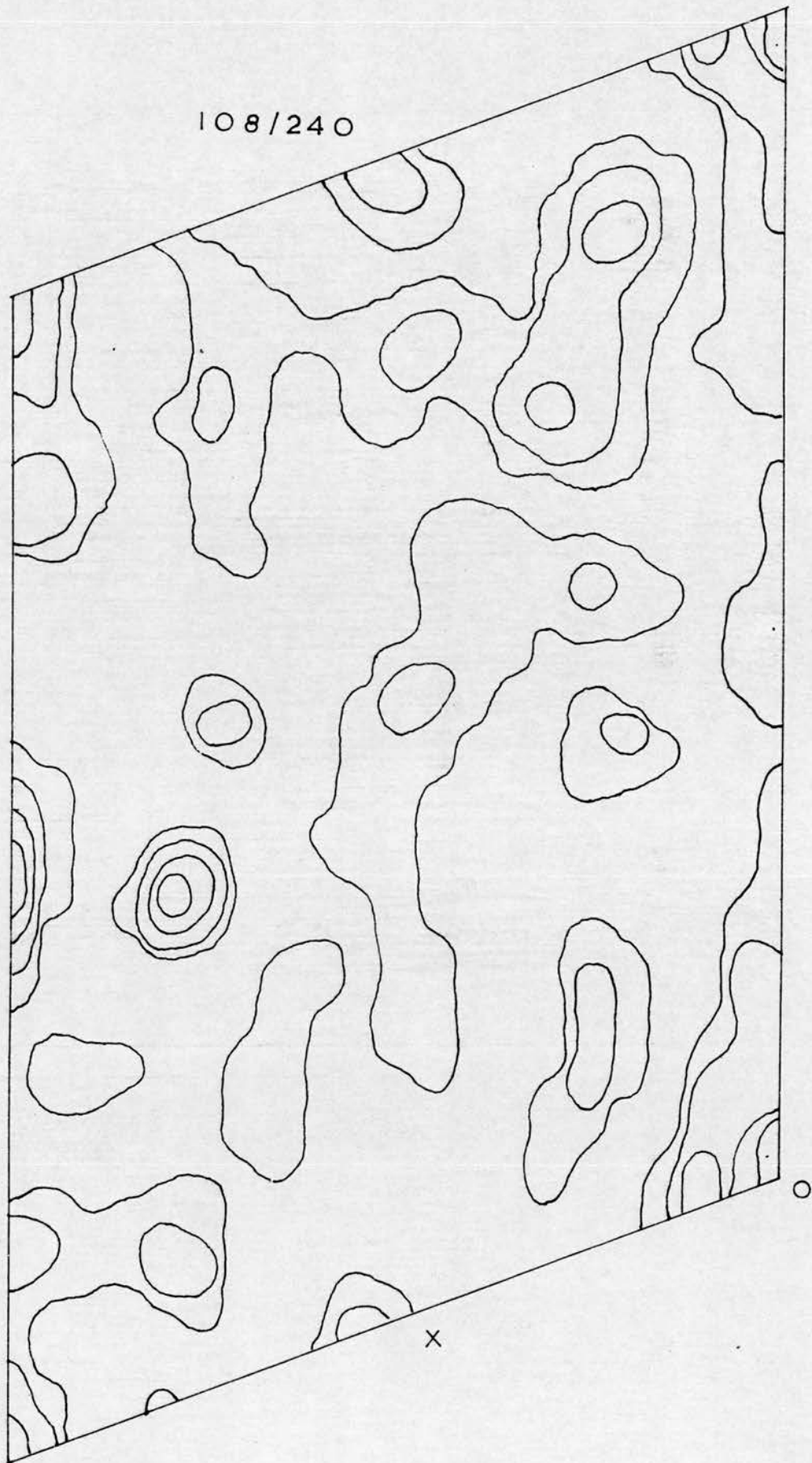


Z

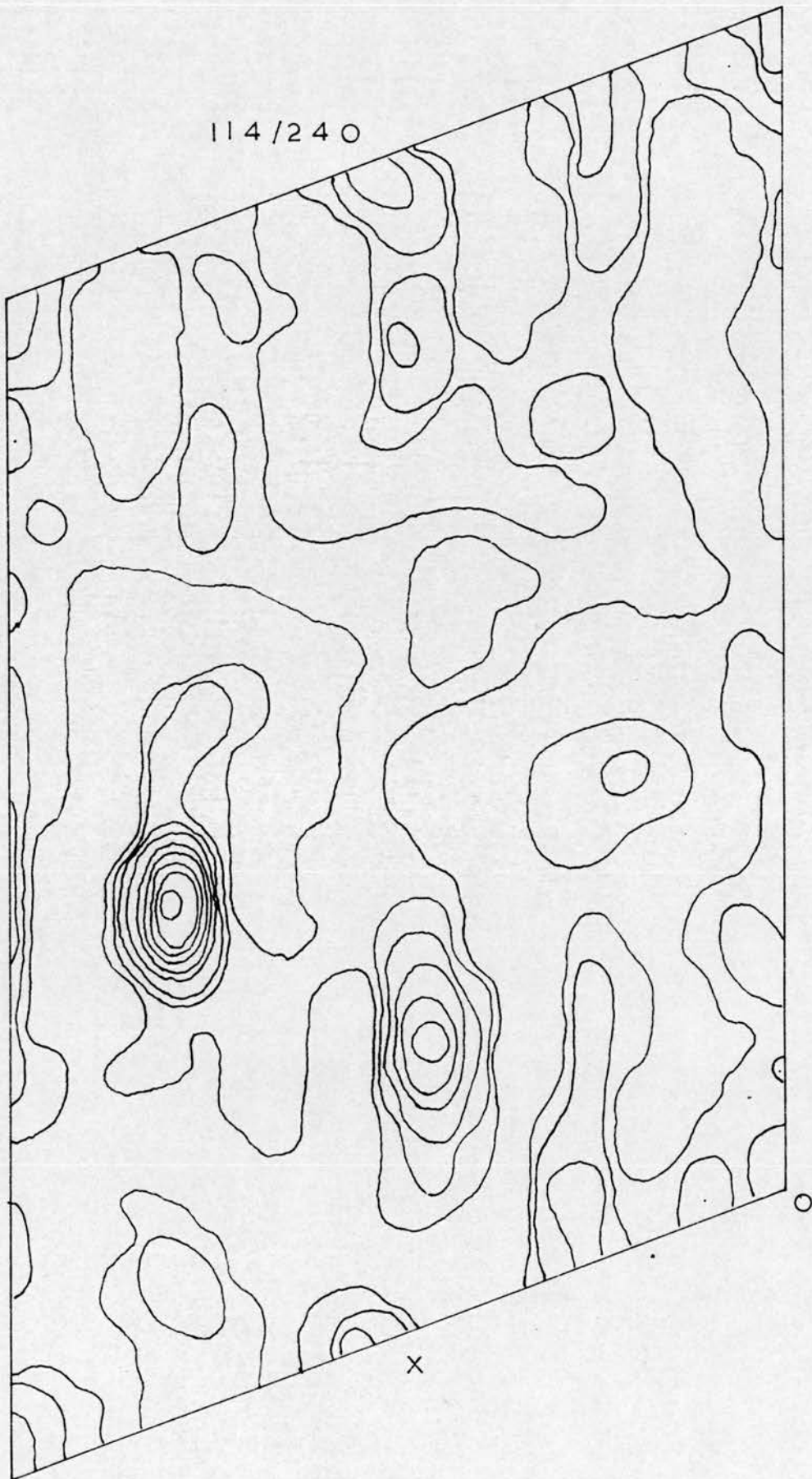
X

○

108/240



114/240



Z

X

0

120/240



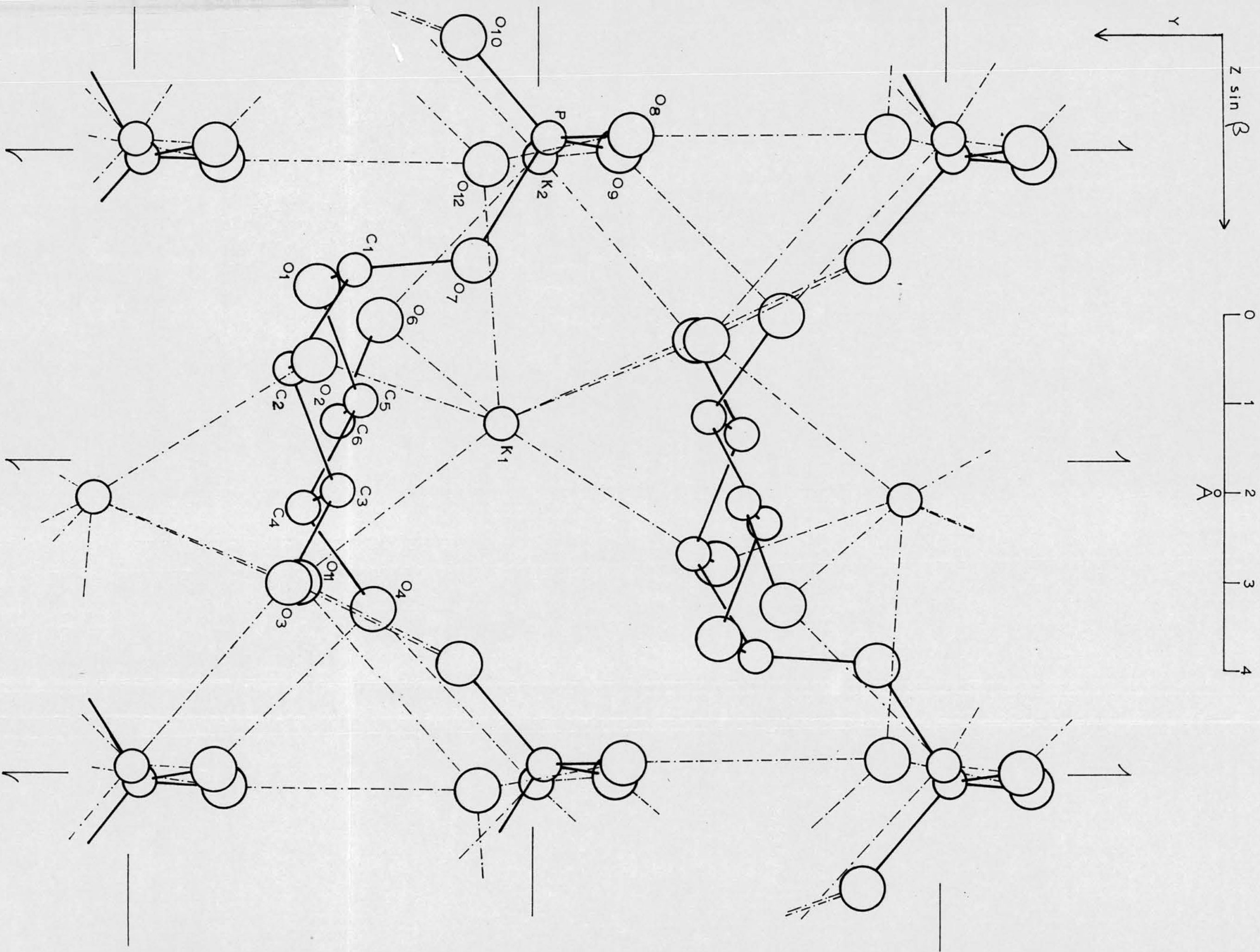
Z

X

o

APPENDIX (3).

Final structure viewed along the a-axis.



APPENDIX (4).

Final structure viewed along the screw axis.

Å

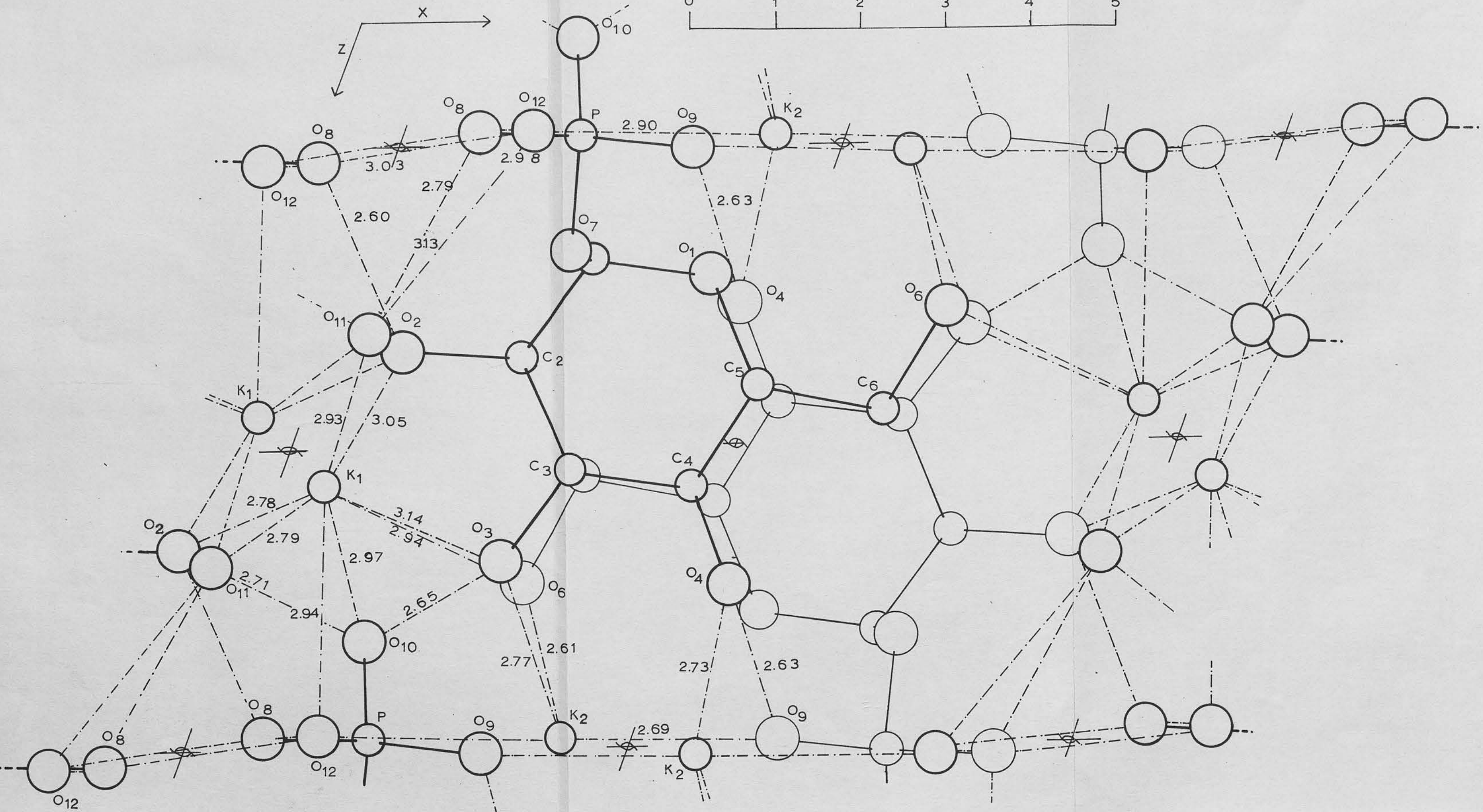
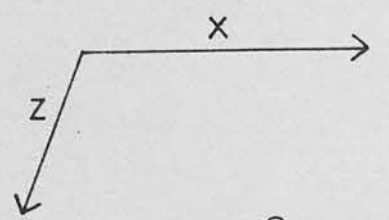
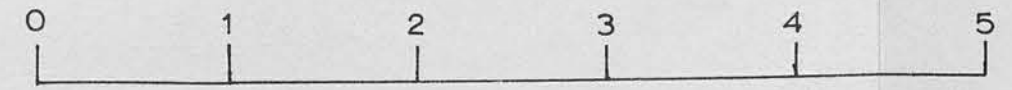


TABLE (2)

Observed and calculated structure factors for

Di potassium Glucose-1-Phosphate, listed as:

L, H

K 10 F(calc) 10F (obs) 1000 cos α 1000 sin α etc.

0, 0
1 384
2 825
3 0
4 2570
5 50
6 490
7 17
8 3330
9 45
10 159
11 0
12 0

0, 1
1 2910
2 1530
3 5990
4 915
5 1590
6 1670
7 2655
8 1160
9 391
10 175
11 395
12 79

0, 2
0 4690
1 5360
2 141
3 4570
4 373
5 5760
6 565
7 480
8 8710
9 175
10 243
11 0
12 51

0, 3
1 2910
2 689
3 4520
4 1750
5 208
6 17
7 74
8 695
9 599
10 96

11 433
12 28

0, 4
0 17800
1 4970
2 774
3 17
4 288
5 1160
6 570
7 130
8 1410
9 264
10 531
11 0

0, 5
1 2540
2 3250
3 972
4 34
5 1500
6 3330
7 938
8 45
9 29
10 34
11 220

0, 6
0 1160
1 39
2 170
3 582
4 162
5 3280
6 339
7 147
8 293
9 219
10 158

0, 7
1 1380
2 605
3 237
4 711
5 203
6 97
7 219
8 170
9 124

0, 8
0 604
1 413
2 610
3 547
4 272
5 151
6 141
7 74
8 124
9 39

0, 9
1 344
2 802
3 944
4 159
5 208
6 479
7 118
8 11
0, 10
0 1020
1 68
2 164
3 266
4 85
5 361
6 39

0, 11
1 162
2 198
3 0
4 56

1, 0
-12 249
-11 88
-10 0
-9 481
-8 1726
-7 1079
-6 95
-5 0
-4 9130
-3 5312
-2 282
-1 2600
0 1250
1 5146
2 1610
3 4150
4 16600

5 0
6 963
7 2496
8 232
9 631

1, 1
-12 415
-11 304
-10 186
-9 531
-8 600
-7 249
-6 192
-5 4117
-4 438
-3 4847
-2 4515
0 4640
0 8130
1 4980
2 4490
3 332
4 1830
5 5310
6 681
7 1830
8 631
9 465
10 996
11 30

1, 2
-12 33
-11 398
-10 179
-9 1430
-8 847
-7 1290
-6 2090
-5 465
-4 581
-3 96
-2 1030
-1 22300
0 7250
1 10700
2 16400
3 631
4 2920
5 531
6 26
7 1830

8 398
9 1360
10 93
11 86

1, 3
-12 435
-11 249
-10 100
-9 128
-8 315
-7 1260
-6 767
-5 963
-4 1450
-3 4600
-2 332
-1 730
0 1290
1 1010
2 126
3 3520
4 2840
5 5480
6 764
7 183
8 282
9 498
10 365
11 26

1, 4
-12 48
-11 373
-10 63
-9 382
-8 1000
-7 299
-6 2660
-5 581
-4 5310
-3 1580
-2 1330
-1 1330
0 100
1 332
2 1660
3 282
4 498
5 2130
6 136

7 191
8 216
9 382
10 0
11 0

1, 5
-11 33
-10 209
-9 432
-8 788
-7 1990
-6 373
-5 606
-4 657
-3 1590
-2 681
-1 2510
0 129
1 2210
2 3320
3 1310
4 1660
5 747
6 96
7 123
8 282
9 126
10 548
11 3

1, 6
-11 66
-10 23
-9 199
-8 888
-7 232
-6 1990
-5 154
-4 996
-3 0
-2 63
-1 1210
0 2420
1 681
2 166
3 863
4 315
5 481
6 266
7 664

8 465
9 1240

1, 7
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-8 48
-7 216
-6 237
-5 1000
-4 1260
-3 1230
-2 91
-1 598
0 448
1 183
2 1860
3 946
4 2070
5 2060
6 432
7 35
8 332
9 8

1, 8
-9 270
-8 0
-7 0
-6 681
-5 30
-4 81
-3 63
-2 96
-1 1590
0 0
1 266
2 481
3 481
4 1780
5 481
6 166
7 81
8 146

1, 9
-8 56
-7 0
-6 189
-5 0
-4 224
-3 432

-2 88
-1 76
0 448
1 448
2 564
3 600
4 58
5 0
6 166
7 166

1, 10
-6 0
-5 26
-4 85
-3 0
-2 0
-1 780
0 0
1 0
2 0
3 83
4 232
5 21

1, 11
-5 7
-4 40
-3 41
-2 103
-1 0
0 199
1 78
2 373
3 13
4 26

2, 0
-12 168
-11 54
-10 1100
-9 405
-8 2680
-7 144
-6 1800
-5 85
-4 2740
-3 270
-2 900
-1 1400
0 5200

-9	216
-8	84
-7	0
-6	1200
-5	190
-4	950
-3	190
-2	96
-1	1200
0	360
1	640
2	144
3	176
4	188
5	8
8	0
-11	30
-10	60
-9	166
-8	96
-7	510
-6	8
-5	227
-4	350
-3	330
-2	140
-1	220
0	69
1	600
2	110
3	108
8	1
-10	0
-9	352
-8	284
-7	66
-6	360
-5	76
-4	62
-3	228
-2	296
-1	40
0	236

1	90
2	120
3	58
9	0
-9	112
-8	198
-7	108
-6	97
-5	0
-4	45
-3	240
-2	40
-1	77
0	300
9	1
-8	164
-7	22
-6	210
-5	180
-4	108
-3	22
-2	60
-1	164
0	16

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4	896	765	841	-541
6	72	94	-702	-712
8	64	82	482	-876
10	225	157	800	600

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4	544	540	-525	-851
5	325	324	-46	999
6	150	103	-925	-379
7	189	152	711	-704
8	152	132	-826	-564
9	105	93	406	-914
10	94	99	-978	207
11	98	98	697	-717

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2	8	77	715	-700
3	126	149	998	-58
4	140	134	955	-297
5	352	314	581	-814
6	114	132	-37	-999
7	174	175	529	-849
8	148	132	-432	-902
9	232	195	546	-838
10	62	55	-716	-698
11	112	117	-52	-999

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1	734	775	437	900
2	404	423	-779	627
3	570	535	434	901
4	39	30	-921	-390
5	346	233	331	944
6	101	112	-866	-500
7	32	82	609	793
8	188	128	-441	-898
9	116	122	-428	904
10	101	94	-775	-633
11	53	36	-992	-128

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2	150	155	-852	-524
3	317	300	884	467
4	101	87	997	83
5	93	113	996	88
6	59	60	988	-154
7	28	23	-586	810
8	70	83	-449	894
9	106	96	-622	-783
10	31	48	-181	-984
11	9	13	-815	578

0	, 5			
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1	302	315	-351	937
2	446	385	988	-158
3	135	160	925	380
4	241	216	962	-274
5	141	134	130	992
6	288	232	820	573
7	61	91	987	-162
8	153	112	879	476
9	38	30	502	865
10	100	137	769	640

0	, 6			
0	215	231	-1000	
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2	138	105	-692	-722
3	34	28	963	269
4	193	207	-768	640
5	286	210	564	-826
6	108	82	-897	-441
7	85	70	824	-566
8	92	82	-446	895
9	147	164	631	-776
10	24	43	-922	-387

0	, 7			
0	109	89	-1000	
1	236	210	999	-56
2	102	88	-310	-951
3	28	28	-177	-984
4	39	27	-960	279
5	145	109	895	-447
6	25	41	997	79

7	129	104	817	-577
8	50	46	-457	890
9	83	86	-333	-943
0, 8				
0	337	246	-1000	
1	291	242	-148	989
2	170	155	-995	-100
3	201	196	-51	999
4	168	150	-983	-184
5	63	51	655	756
6	107	94	-989	146
7	113	85	394	919
8	48	65	-840	542
9	34	30	-391	920
0, 9				
0	16	27	1000	
1	41	27	618	786
2	79	82	85	996
3	107	83	-696	718
4	142	121	248	969
5	71	63	316	-949
6	76	75	926	379
7	45	50	85	996
8	97	68	906	423
0, 10				
0	18	28	-1000	
1	66	56	858	513
2	101	89	295	955
3	63	58	-705	710
4	132	114	513	858
5	22	52	-246	969
6	154	137	892	453
7	23	38	166	986
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1	127	111	-189	-982
2	97	82	-782	624
3	169	133		-1000
4	78	62	-695	719
5	127	109	-46	-999
0, 12				
0	4	29	-1000	
1	47	41	240	-971
2	44	30	-941	337
3	37	43	-981	-192

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1, -12				
0	53	59	1000	
1	49	49	-997	-79
2	34	33	535	-845
3	40	29	744	-668
1, -11				
0	8	24	1000	
1	156	116	186	983
2	15	29	48	-999
3	114	106	121	993
1, -10				
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1	82	98	-917	-399
2	144	144	948	320
3	56	75	955	-296
4	132	110	683	-731
1, -9				
0	192	187	1000	
1	48	59	773	-634
2	190	150	992	-128
3	24	23	90	-996
4	69	60	692	722
1, -8				
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1	238	207	241	-971
2	195	152	-963	-269
3	127	127	676	-737
4	107	91	-217	-976
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1	260	259	543	840
2	60	88	306	-952
3	203	212	301	954
4	90	110	395	-919
1, -6				
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1	508	480	510	860
2	124	168	-940	340
3	433	395	251	968
4	277	256	-672	-740

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0	738	689	1000		0	410	464	-1000	
1	219	258	271	963	1	487	544	430	-903
2	176	260	-315	-949	2	59	61	447	894
3	322	301	873	-487	3	504	500	-324	-946
4	418	375	999	-34	4	202	185	-855	519
1, -4					1, 3				
0	544	480	-1000		0	522	498	-1000	
1	238	275	315	949	1	98	118	-971	241
2	118	78	119	993	2	110	129	954	302
3	295	316	-422	907	3	362	347	-214	977
4	40	29	-842	-539	4	341	284	-831	556
1, -3					1, 4				
0	320	264	-1000		0	94	106	-1000	
1	491	486	-140	-990	1	500	407	193	-981
2	905	763	-860	510	2	185	195	-839	-544
3	80	117	286	-958	3	203	250	-1000	
4	302	232	-558	830	4	166	147	478	878
1, -2					1, 5				
0	457	372	-1000		0	68	83	1000	
1	481	475	400	916	1	84	49	-348	-938
2	582	574	-796	605	2	364	326	1000	
3	161	170	-197	981	3	233	228	-911	-412
4	170	140	-199	980	4	314	316	999	-42
1, -1					1, 6				
0	191	101	-1000		0	189	132	-1000	
1	274	293	-149	989	1	237	212	-108	-994
2	620	481	-989	151	2	171	158	771	-637
3	155	180	-938	-346	3	181	200	57	-998
1, 0					1, 7				
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1	250	279	-805	-593	1	181	170	633	-774
2	977	642	984	178	2	185	164	-58	-998
3	93	81	702	-712	3	107	127	-250	-968
4	335	342	-172	985	4	243	246	534	-846
5	205	255	-972	-236	1, 8				
6	187	188	994	106	0	1	23	-1000	
7	24	23	-967	257	1	250	241	-565	825
8	282	250	866	500	2	162	132	-390	-921
9	85	65	-485	874	3	99	109		1000
10	198	190	912	410	4	214	152	-502	-865
1, 1					1, 9				
0	166	93	-1000		0	108	106	1000	
1	409	442	481	-877	1	99	78	-715	700
2	358	301	-1000		2	145	102	979	205
3	41	30	-345	939	3	43	66	-736	-677
4	331	158	-757	654	4	71	86	672	-741

1 , 10					2 , -7				
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1	106	97	-586	811	1	59	52	940	-342
2	107	93	993	122	2	61	51	749	-663
3	87	121	667	745	3	206	200	-811	-585
4	141	89	968	250	4	62	92	486	-874
1 , 11					2 , -6				
0	10	27	1000		0	42	39	1000	
1	79	70	707	-707	1	123	131	-85	996
2	36	28	-1000		2	96	154	-737	-676
3	104	70	38	-999	3	156	174	45	999
1 , 12					4	113	121	-526	-850
0	32	38	1000		2 , -5				
2 , -13					0	299	258	1000	
0	88	79	-1000		1	241	268	65	-998
1	38	37		-1000	2	532	510	883	-469
2 , -12					3	159	121	324	-946
0	25	26	-1000		4	229	240	978	-211
1	25	21	825	565	2 , -4				
2	120	75	-777	630	0	34	29	1000	
3	20	17	985	-174	1	255	277	-759	651
2 , -11					2	248	294	612	-791
0	94	71	-1000		3	142	205	-368	-930
1	166	162	72	997	4	108	126	944	-328
2	105	125	-887	461	2 , -3				
3	156	118	261	965	0	259	275	-1000	
4	85	51	-898	440	1	458	502	-182	-983
2 , -10					2	149	199	977	-212
0	21	23	1000		3	386	408	18	-999
1	109	115	748	-664	4	223	173	-990	-144
2	73	60	998	62	2 , -2				
3	120	130	-848	-530	0	149	208	-1000	
4	132	124	345	939	1	114	224	-217	976
2 , -9					2	543	549	842	-540
0	188	127	1000		3	401	386	-228	974
1	242	173	185	-983	4	130	130	-710	-704
2	169	141		1000	2 , -1				
3	141	132	-874	486	0	194	234	1000	
4	106	120	998	-64	1	360	355	-529	849
2 , -8					2	322	254	-603	-798
0	190	147	-1000		3	253	237	-994	-111
1	435	373	-130	-992	4	114	278	-386	-923
2	310	331	-1000		2 , 0				
3	229	205	-248	-969	0	452	419	1000	
4	113	109	-929	369	1	200	194	-187	982
					2	999	872	987	161

3	128	111	929	-371
4	238	180	993	116
5	64	125	644	765
6	182	191	811	-585
7	56	77	883	-470
8	220	214	986	-164
9	22	24	-996	-95
10	52	71	504	864
11	27	85	900	-435

2, 1

0	53	69	-1000	
1	378	397	887	461
2	201	195	661	-750
3	552	546	739	674
4	163	139	-923	384

2, 2

0	463	452	1000	
1	274	331	770	638
2	172	167	-993	-114
3	409	380	700	-714
4	216	211	-92	-996

2, 3

0	29	47	1000	
1	414	371	-187	982
2	148	115	-947	321
3	453	412	424	906
4	197	190	-986	-169

2, 4

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1	87	78	-472	-882
2	193	252	-867	499
3	141	155	802	598
4	62	93	-292	957

2, 5

0	42	24	1000	
1	119	118	-435	901
2	125	127	427	904
3	136	195	101	995
4	293	299	837	547

2, 6

0	30	37	1000	
1	135	133	981	-192
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3	208	246	141	-990
4	41	23	833	-554

2, 7

0	167	105	-1000	
1	261	305	-938	-374
2	51	55	203	-979
3	299	287	852	-523
4	41	28	-890	-457

2, 8

0	199	198	-1000	
1	161	147	200	980
2	200	161	-924	-382
3	141	158	-743	669
4	143	122	-976	217

2, 9

0	20	24	1000	
1	13	24	-114	994
2	94	77	-127	992
3	62	64	-991	131

2, 10

0	72	72	1000	
1	33	31	-754	657
2	69	53	795	607
3	33	39	-753	658

3, -13

0	25	24	-1000	
1	45	42	-242	-970
2	54	37	-553	-833

3, -12

0	12	26	-1000	
1	49	36	932	362
2	48	37	969	246
3	27	28	939	-345

3, -11

0	29	24	1000	
1	213	209	34	999
2	16	24	-749	-663
3	202	211	444	896
4	53	51	-392	-920

3, -10

0	164	51	1000	
1	60	46	268	964
2	79	70	999	-28
3	40	23	871	-492
4	86	86	976	218

3 , -9				
0	119	109	1000	
1	28	55	-281	960
2	184	155	954	301
3	68	110	967	254
4	108	87	770	638

3 , -8				
0	248	198	-1000	
1	173	189	-287	-958
2	169	133	-961	-276
3	197	232	852	-523
4	138	150	-996	-85

3 , -7				
0	139	118	-1000	
1	123	135	-51	999
2	126	107	682	-731
3	39	116	349	937
4	71	37	-996	-86

3 , -6				
0	266	281	-1000	
1	337	322	55	999
2	139	166	-960	-280
3	148	212	560	829
4	215	233	-652	758

3 , -5				
0	284	265	-1000	
1	83	59	966	-258
2	266	276	915	403
3	194	202	986	167
4	234	214	757	653

3 , -4				
0	89	85	-1000	
1	349	366	-132	991
2	353	375	-928	372
3	205	189	802	598
4	214	209	257	967

3 , -3				
0	600	610	-1000	
1	127	110	652	-758
2	104	120	-723	691
3	549	599		-1000
4	424	369	-949	315

3 , -2				
0	85	75	1000	
1	165	165	-765	-644
2	412	380	-998	-66
3	357	365	-655	755
4	95	97	970	-243

3 , -1				
0	271	283	-1000	
1	404	410	-898	-441
2	490	486	-948	318
3	371	369	-997	-80
4	190	175	-991	-131

3 , 0				
0	612	610	1000	
1	279	248	-360	-933
2	468	446	479	878
3	261	260	-839	-544
4	272	226	840	542
7	174	177	-781	625
8	110	78	984	-176
9	27	24	177	984
10	202	200	945	326
11	83	88	-294	956

3 , 1				
0	204	233	1000	
1	59	78	969	-249
2	311	295	-784	621
3	324	354	262	-965
4	211	157	620	-785

3 , 2				
0	41	35	1000	
1	452	460	-419	-908
2	145	257	530	-848
3	258	269	549	-836
4	96	108	-879	-477

3 , 3				
0	302	272	1000	
1	152	155	855	518
2	172	214	-992	127
3	157	193	-738	675
4	161	163	-649	-761

3 , 4				
0	93	88	-1000	
1	169	170	-439	-898
2	32	55		1000
3	17	23	989	-146
4	129	124	-181	-984

3 , 5				
0	279	244	1000	
1	26	51	-982	188
2	295	317	912	-410
3	113	100	-858	514
4	271	205	998	70

3, 6
 0 26 23 1000
 1 156 141 -1000
 2 116 77 708 -706
 3 69 96 324 -946
 4 57 110 -441 -898

3, 7
 0 28 23 1000
 1 180 170 614 -790
 2 115 138 637 -771
 3 77 102 -373 -928
 4 106 85 305 -952

3, 8
 0 150 103 -1000
 1 196 216 95 996
 2 76 62 -698 -717
 3 142 142 -69 998
 4 122 77 -686 -728

3, 9
 0 93 97 1000
 1 124 93 337 941
 2 33 26 778 -628
 3 68 49 250 968

3, 10
 0 113 70 1000
 1 111 95 495 871
 2 104 36 993 -121

4, -13
 0 83 96 -1000
 1 90 85 -864 -504
 2 66 37 -991 -131

4, -12
 0 42 42 -1000
 1 43 42 -304 -954
 2 49 32 -578 816
 3 74 72 -152 -988

4, -11
 0 104 86 -1000
 1 109 96 -60 998
 2 50 49 -905 426
 3 84 100 -470 883
 4 53 94 -733 680

4, -10
 0 42 23 1000
 1 108 121 -794 -608
 2 99 107 33 999
 3 68 85 -629 -778
 4 77 67 871 492

4, -9
 0 214 146 1000
 1 122 114 -774 -632
 2 96 105 848 530
 3 95 133 464 -886
 4 162 130 712 703

4, -8
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 1 364 359 501 -865
 2 124 123 675 -738
 3 224 264 -524 -852
 4 214 250 -924 -382

4, -7
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 1 189 191 -368 -930
 2 181 167 -638 -770
 3 137 156 -995 103
 4 246 255 450 -893

4, -6
 0 109 66 1000
 1 83 66 -228 974
 2 24 88 -836 -549
 3 182 256 -677 736
 4 168 193 -533 -846

4, -5
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 1 92 82 466 -885
 2 246 266 999 32
 3 67 102 788 -615
 4 344 313 964 -266

4, -4
 0 84 73 1000
 1 363 369 352 936
 2 150 240 999 -55
 3 78 124 935 354
 4 251 214 780 -625

4, -3
 0 383 374 1000
 1 215 287 612 -791
 2 305 310 -994 -112
 3 139 95 965 -261
 4 144 168 -249 -969

4, -2
 0 184 175 1000
 1 295 317 -224 975
 2 117 63 327 -945
 3 80 43 967 255
 4 196 197 -73 -997

4 , -1					4 , 6				
0	317	203	-1000		0	99	124	-1000	
1	346	274	-631	776	1	208	233	-359	-933
2	24	87	-989	-145	2	33	60	-979	205
3	259	250	-177	984	3	45	60	-388	-922
4	228	207	-881	-473	4	80	67	-668	745
4 , 0					4 , 7				
0	574	417	1000		0	107	74	-1000	
1	176	165	748	663	1	208	200	217	-977
2	284	288	971	237	2	44	58	-905	-427
3	127	176	-87	996	3	166	144	-480	-877
4	251	256	846	533	4	43	52	53	999
5	106	147	972	-234	4 , 8				
6	121	200	924	-383	0	141	93	-1000	
7	79	152	961	-278	1	104	94	-282	959
8	95	188	957	291	2	167	154	-980	197
9	15	26	-522	-853	3	47	72	-912	411
10	149	150	820	573	4 , 9				
4 , 1					0	9	89	1000	
0	92	82	-1000		1	70	60	-863	505
1	165	134	641	768	2	17	12	674	-739
2	85	62	1000		5 , -13				
3	208	301	990	140	0	17	31	-1000	
4	64	120	-981	194	1	53	50	983	-182
4 , 2					5 , -12				
0	189	207	-1000		0	96	119	1000	
1	139	52	475	-880	1	31	38	-956	295
2	75	60	-756	654	2	43	33	-818	-575
3	191	163	504	-864	3	60	52	673	-740
4	121	133	-780	625	5 , -11				
4 , 3					0	43	34	-1000	
0	272	242	-1000		1	183	196	238	971
1	253	298	434	901	2	49	35	-988	153
2	175	184	-747	664	3	145	149	71	998
3	296	331	282	960	4	16	32	206	-979
4	253	191	-701	714	5 , -10				
4 , 4					0	22	23	-1000	
0	301	235	-1000		1	59	66	366	931
1	130	106	920	392	2	101	85	171	985
2	129	137	-999	-37	3	86	77	267	964
3	119	123	623	782	4	88	74	751	660
4	212	168	-674	739	5 , -9				
4 , 5					0	109	83	1000	
0	109	73	1000		1	149	175	939	344
1	76	96	845	535	2	175	143	564	826
2	125	141	996	90	3	87	140	456	890
3	103	138	-860	510	4	108	80	581	814
4	161	167	984	181					

5, -8				
0	18	23	1000	
1	154	132	429	-903
2	332	264	-1000	
3	127	159	665	-747
4	39	46	-882	-472
5, -7				
0	169	147	-1000	
1	25	23	-468	-884
2	39	36	-335	-942
3	82	50	-777	-629
4	110	109	-999	31
5, -6				
0	231	207	-1000	
1	100	51	-212	977
2	199	244	-956	293
3	268	341	-66	998
4	165	146	-953	302
5, -5				
0	68	74	1000	
1	105	84	796	-605
2	24	23	-993	119
3	108	110	444	-896
4	221	209	881	472
5, -4				
0	239	263	-1000	
1	113	99	-216	976
2	152	126	999	-31
3	74	129	-476	-879
4	120	120	456	890
5, -3				
0	315	291	-1000	
1	366	306	-267	-964
2	149	195	-945	326
3	229	313	-128	-992
4	247	184	-677	736
5, -2				
0	208	127	-1000	
1	81	71	-787	-617
2	94	102	-1000	
3	88	121	-980	-198
4	98	118	-154	988
5, -1				
0	386	329	-1000	
1	109	118	-535	845
2	72	79	146	-989
3	202	276	-996	-94
4	272	281	-995	102

5, 0				
0	487	377	1000	
1	106	121	-13	-999
2	370	344	995	-101
3	173	258	-879	-477
4	165	173	999	-52
5	61	61	-999	52
6	37	23	-538	-843
7	93	95	-957	290
8	123	179	963	271
9	57	58	-473	881
5, 1				
0	191	102	1000	
1	209	265	-371	-929
2	204	152	796	-605
3	43	118	-753	658
4	54	68	484	-875
5, 2				
0	56	28	1000	
1	250	253	90	-996
2	33	23	-71	998
3	300	328	-351	-936
4	82	109	-46	-999
5, 3				
0	99	27	-1000	
1	161	182	-458	889
2	89	82	502	865
3	129	141	726	688
4	98	92	-689	-725
5, 4				
0	70	64	1000	
1	136	150	931	365
2	136	152	-777	-630
3	106	93	455	890
4	38	28	930	365
5, 5				
0	259	265	1000	
1	113	116	-591	807
2	195	185	997	-80
3	79	124	879	478
4	184	186	764	-645
5, 6				
0	65	29	1000	
1	53	44	-564	-826
2	34	25	501	-865
3	66	82	484	-875
5, 7				
0	2	27	-1000	
1	85	70	-97	-995
2	39	22	-152	-988
3	98	75	532	-847

5, 8
0 92 49 -1000
1 134 104 -202 979

6, -12
1 101 111 164 -986

6, -11
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1 78 77 -429 903
2 34 74 -971 239
3 92 99 -150 989

6, -10
0 77 72 1000
1 41 73 -951 310
2 56 39 562 827
3 54 91 -517 -856
4 69 48 979 206

6, -9
0 152 107 1000
1 58 51 193 -981
2 221 240 984 178
3 38 24 -984 177
4 99 88 981 195

6, -8
0 46 37 -1000
1 231 235 -213 -977
2 100 101 -689 -725
3 130 152 185 -983
4 58 67 -911 -414

6, -7
0 9 18 1000
1 52 68 -793 -609
2 86 132 233 -973
3 125 115 -75 -997
4 61 65 -53 -999

6, -6
0 96 132 -1000
1 210 205 300 954
2 126 150 659 -752
3 219 232 214 977
4 166 175 -942 -335

6, -5
0 200 182 1000
1 83 50 690 724
2 306 314 828 -561
3 109 114 999 44
4 138 184 910 -415

6, -4
0 203 164 1000
1 148 146 -236 972
2 146 123 800 -600
3 107 168 74 997
4 161 132 925 -380

6, -3
0 192 170 -1000
1 94 97 -36 -999
2 132 105 -826 564
3 183 287 236 -972

6, -2
0 339 271 -1000
1 138 193 41 999
2 47 91 942 335
3 170 287 532 847
4 94 91 -745 668

6, -1
0 115 118 1000
1 337 323 399 917
2 409 382 -999 31
3 123 150 -479 878
4 22 23 -998 70

6, 0
0 236 232 1000
1 170 196 -819 574
2 246 241 927 375
3 112 159 991 -132
4 30 46 973 -233
5 41 102 -738 675
6 127 197 275 962
7 44 73 629 -778
8 114 240 912 410

6, 1
0 33 23 1000
1 34 23 -940 -340
2 27 82 -949 315
3 37 32 946 323
4 43 70 125 992

6, 2
0 97 110 -1000
1 157 170 -33 -999
2 157 129 -759 651
3 180 144 116 -993
4 42 56 -992 126

6, 3
 0 42 23 -1000
 1 102 107 -491 871
 2 189 193 -856 517
 3 82 133 220 976
 4 99 93 -651 759

6, 4
 0 158 43 -1000
 1 53 67 -996 89
 2 139 146 -848 532
 3 61 65 989 147
 4 67 62 -999 -30

6, 5
 0 75 51 1000
 1 36 26 -911 -412
 2 69 95 997 -79
 3 50 65 -929 370

6, 6
 0 49 51 -1000
 1 107 74 492 -871
 2 20 12 328 945

7, -11
 0 57 51 -1000
 1 162 207 -162 987
 2 67 47 -950 311

7, -10
 0 42 26 -1000
 1 89 74 -96 995
 2 43 54 943 334
 3 12 22 860 -512

7, -9
 0 131 98 1000
 1 32 29 -379 -925
 2 99 75 609 793
 3 8 21 652 759
 4 137 124 809 588

7, -8
 0 218 291 -1000
 1 189 214 31 -999
 2 144 125 -910 414
 3 118 103 224 -975
 4 94 102 -639 770

7, -7
 0 143 112 -1000
 1 91 93 133 -991
 2 99 103 -848 531
 3 31 57 -971 -240
 4 90 39 -905 426

7, -6
 0 36 23 1000
 1 161 156 317 948
 2 223 279 -990 138
 3 51 24 -250 968
 4 64 67 -994 -84

7, -5
 0 25 23 1000
 1 105 124 -132 -991
 2 132 207 934 -356
 3 12 74 211 977
 4 83 80 927 -374

7, -4
 0 153 109 1000
 1 58 68 -655 -756
 2 35 37 497 -868
 3 95 121 -979 206
 4 131 127 999 -55

7, -3
 0 85 59 -1000
 1 218 258 -29 -999
 2 124 198 -745 667
 3 182 242 -300 -954
 4 64 80 -934 -357

7, -2
 0 32 64 1000
 1 20 23 -99 995
 2 74 87 -979 -206
 3 86 98 -945 -328
 4 98 108 951 -308

7, -1
 0 116 97 -1000
 1 166 175 -862 507
 2 49 60 -481 -877
 3 67 38 -883 470
 4 116 105 -880 -475

7, 0
 0 296 274 1000
 1 50 56 124 -992
 2 186 200 996 -86
 3 88 105 -997 -79
 4 120 112 972 236
 5 63 51 -959 -83
 6 58 144 876 -482
 7 63 62 -881 732

7, 1
 0 84 56 1000
 1 52 47 995 102
 2 135 133 999 29
 3 27 19 701 -713
 4 77 66 477 -879

7, 2
 0 33 63 1000
 1 88 116 -200 -980
 2 68 84 705 -709
 3 127 133 489 -872
 4 62 54 -743 -670

7, 3
 0 68 56 1000
 1 99 103 593 805
 2 49 53 -29 -999
 3 110 109 -45 998

7, 4
 0 76 67 -1000
 1 33 28 655 756
 2 28 110 -931 -361

7, 5
 0 27 19 1000
 1 54 37 -45 998

8, -11
 0 33 38 -1000

8, -10
 0 112 119 1000
 1 22 36 695 719
 2 34 31 959 283

8, -9
 0 232 234 1000
 1 20 22 194 -981
 2 111 102 940 342
 3 14 41 423 907

8, -8
 0 62 52 -1000
 1 98 104 110 -994
 2 45 69 -975 221
 3 73 77 486 -874

8, -7
 0 38 45 1000
 1 21 25 724 -690
 2 60 41 -873 -489
 3 20 27 302 -953
 4 70 50 166 -986

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 1 143 181 263 965
 2 44 64 -377 -926
 3 170 209 295 956
 4 97 64 -948 -319

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 1 61 68 821 570
 2 53 78 866 -491
 3 71 71 939 343
 4 89 89 1000

8, -4
 0 21 24 1000
 1 137 192 39 999
 2 73 92 976 218
 3 89 100 652 758
 4 94 68 999 -20

8, -3
 0 153 125 -1000
 1 47 73 464 -886
 2 43 29 -878 -478
 3 84 110 943 -334
 4 128 89 -758 652

8, -2
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 1 54 83 781 625
 2 73 94 -768 -641
 3 27 51 -807 590
 4 24 27 184 983

8, -1
 0 214 79 -1000
 1 105 135 -618 787
 2 97 106 -954 301
 3 91 84 -695 719
 4 103 66 -972 235

8 , 0				
0	7	31	1000	
1	13	15	165	-986
2	115	137	804	594
3	48	55	-905	425
4	164	226	173	985
5	52	62	-844	-537

8 , 1				
0	2	11	1000	
1	56	72	897	443
2	46	73	-254	967

8 , 2				
0	11	12	-1000	
1	101	146	-47	-999
2	51	30	-949	-317

8 , 3				
0	29	31	1000	
1	29	19	-972	-234

9 , -8				
0	109	85	-1000	
1	113	129	72	-997

9 , -7				
0	28	146	-1000	
1	86	89	-110	-994
2	26	30	-835	-550

9 , -6				
0	66	29	-1000	
1	29	23	-987	164
2	31	31	-998	68

9 , -5				
0	36	134	1000	
1	57	73		-1000
2	29	23	564	-826

9 , -4				
0	6	39	1000	
1	53	85	-172	985
2	144	151	996	-93
3	24	18	-113	-994

9 , -3				
0	42	40	1000	
1	107	137	429	-903
2	49	70	-429	-903
3	57	18	-28	-919

9 , -2				
0	16	23	1000	
1	47	65	-692	-722
2	62	67	508	-861

9 , -1				
0	42	24	-1000	
1	77	43	-518	855
2	67	50	-933	-361

9 , 0				
0	126	120	1000	
1	37	32	42	999

TABLE (3)

Relative F^2 's of Di ammonium Glucose-1-Phosphate

listed as

L, K

H F^2

etc.

1 5940
2 121
3 2320
4 562
5 1440
6 720
7 1100
8 81
9 0
10 45

2, 1
-13 140
-12 54
-11 101
-10 405
-9 2020
-8 540
-7 220
-6 292
-5 1570
-4 1260
-3 2250
-2 1010
-1 4400
0 1240
1 2740
2 1710
3 517
4 1390
5 220
6 4500
7 2430
8 415
9 742
10 0

2, 2
-12 67
-11 63
-10 877
-9 144
-8 1480
-7 472
-6 495
-5 337
-4 765
-3 2560
-2 23600
-1 1550
0 6970

1 225
2 1080
3 2540
4 1510
5 0
6 3730
7 207
8 585
9 47
10 49

2, 3
-12 31
-11 72
-10 281
-9 2860
-8 1170
-7 675
-6 216
-5 1910
-4 4840
-3 3940
-2 47
-1 2860
0 3370
1 2520
2 585
3 200
4 1440
5 945
6 585
7 247
8 243
9 427

2, 4
-12 63
-11 0
-10 337
-9 405
-8 506
-7 2250
-6 83
-5 1660
-4 967
-3 742
-2 765
-1 36
0 774
1 1780
2 495

3 1390
4 405
5 38
6 61
7 405
8 256
9 0
10 112

2, 5
-10 58
-9 225
-8 405
-7 0
-6 2250
-5 2020
-4 1860
-3 2500
-2 247
-1 1550
0 256
1 1080
2 58
3 929
4 382
5 81
6 101
7 270
8 42
9 85

2, 6
-10 54
-9 0
-8 326
-7 1620
-6 162
-5 1170
-4 1080
-3 427
-2 1210
-1 1600
0 652
1 675
2 270
3 967
4 607
5 0
6 76
7 236

8 74
9 38
2, 7
-10 22
-9 225
-8 470
-7 112
-6 141
-5 1030
-4 3240
-3 58
-2 0
-1 292
0 585
1 1780
2 304
3 0
4 607
5 94
6 0
7 115
8 56

2, 8
-7 146
-6 191
-5 76
-4 40
-3 101
-2 1210
-1 405
0 315
1 517
2 99
3 765
4 0
5 74
6 216
7 169

2, 9
-6 49
-5 58
-4 67
-3 450
-2 0
-1 184
0 96
1 450
2 83

3	220
4	495
5	34
6	27
2	10
-5	88
-4	101
-3	0
-2	38
-1	52
0	187
1	117
2	0
3	79
4	54
2	11
-3	56
-2	0
-1	27
0	45
1	11
2	9
3	0
-12	189
-11	1000
-10	72
-9	94
-8	1520
-7	298
-6	1100
-5	252
-4	2520
-3	8500
-2	940
-1	109
0	14000
1	97
2	3200
3	940
4	2170
5	2980
6	361
7	1450
8	0
9	53

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-10	1190
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-8	88
-7	273
-6	346
-5	4270
-4	578
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-2	2460
-1	920
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1	3740
2	104
3	3810
4	270
5	2700
6	361
7	192
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9	50
3	2
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-10	0
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-6	377
-5	1730
-4	1820
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-2	5710
-1	4260
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1	4700
2	3920
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7	72
8	0

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-5	3950
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-2	7660
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1	722
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3	879
4	0
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6	72
7	0
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-9	94
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-10	314
-9	330
-8	239

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-6	1420
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-3	0
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-1	34
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2	214
3	4180
4	195
5	119
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7	69
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-10	110
-9	34
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-6	377
-5	471
-4	565
-3	2670
-2	217
-1	1950
0	110
1	1730
2	2140
3	720
4	0
5	113
6	754
3	7
-9	91
-8	34
-7	236
-6	167
-5	47
-4	2170
-3	440
-2	1190
-1	1670
0	6540
1	0

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5	263	-3	800	-1	160	4	380
6	47	-2	1180	0	1460	5	140
		-1	2650	1	8	6	113
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0	119	9	0	-13	106	-6	1100
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6	9	-10	720	-7	1180	0	940
		-9	480	-6	1080	1	288
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-3	75	-4	590	-1	60	6	72
-2	471	-3	2400	0	80		
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		3	336	6	360	-8	360
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2	17	5	0	-9	0	0	2230
		-13	76	-8	745	1	1350
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