

instrument. The availability of good electron microscopes is energetically exploited by Japanese laboratories in various fields of biology, medicine, metallurgy, colloid science, polymer chemistry and crystallography. In biology and medicine the breadth of fields of interest applying the electron microscope is impressive. Active workers using the electron microscope are found in departments of genetics, anatomy, microbiology, physiology, pathology, internal medicine, orthopaedic surgery, dentistry, botany, zoology and virology. The vigour and enthusiasm and breadth of base manifested in this development betokens a bright future for the structural biological sciences in Japan.

The picture of electron microscopy in the Soviet Union presented by Mr. V. G. Nyrikov, director of the Institute of Electronic Optics, Ministry of Radio-technical Industry, U.S.S.R., displayed an active development. Mr. Nyrikov stated that electron microscopes have been manufactured commercially in the Soviet Union since 1946, that about 350 instruments now exist in that country, and that these are of several types. Most models utilize low-frequency high-voltage stabilizers and electro-magnetic lenses, though two electrostatic models are made. Mr. Nyrikov indicated that the resolving capabilities of most of the instruments are rather modest, and micrographs taken with Soviet instruments and shown at the Conference were not impressive from the point of view of quality and resolution. However, Mr. Nyrikov indicated that active attention is being given in the Soviet Union to design and improvement of electron microscopes, and that an experimental instrument with a special stabilizer, capable of a resolution of 20 Å., has been built. Various other representatives from the Soviet Union presented papers relating to applications of the electron microscope to studies in biology and ceramics.

A summary of the current status of electron microscopy in the Peoples Republic of China was presented by Dr. Li Lin, of the Institute of Metallurgy and Ceramics, Academia Sinica, Shanghai. Speaking elegant English, Dr. Li explained that there are very few electron microscopes in China (the number recalled is six) and that electron microscopy is in its infancy there, since the energies of the country are being expended along lines deemed to be more urgent. Dr. Li showed some electron microscope studies in the field of metallurgy, involving examination of slip planes in single crystals of metals induced by shearing

forces imposed on the crystals. No biological work from China was presented, though several workers in biological fields were members of the delegation.

The reports of work emanating from India were impressive for their high quality of microscopy and scholarly excellence. Yet the Conference was told that there are very few electron microscopes in India (the figure of two microscopes in India is recalled, though this may be an error). It is evident that these few instruments are used with skill and imagination.

Electron microscopy in Indonesia was presented by Prof. T. G. F. Schoon, of the Foundation for Rubber Research and Development at Bogor.

An eloquent summary of the Conference was prepared by Prof. B. Prakash, of the Department of Metallurgy, Indian Institute of Science, at Bangalore. With a charming Scottish accent, delightful wit, and discerning cultural understanding, Prof. Prakash spoke of the great stimulus the Conference had provided, of the cordial hospitality and efficient organization of the Japanese hosts, and of the magnificent service to international understanding, scientific exchange and cultural appreciation, which the Conference had rendered. Prof. Prakash stated that a decision has been made to hold the next Regional Conference on Electron Microscopy in Asia and Oceania in India four years hence.

The Proceedings of the Conference are to be published in English under the technical auspices of the *Journal of Electronmicroscopy* and the Society of Electron Microscopy, Japan. English and Japanese were the official languages. The sessions were held in Sankei International Conference Hall, which is equipped with ear-phones and translating booths similar to those used at United Nations meetings.

For many who were present the international scientific contacts established and nourished outside the formal sessions were of special importance, and many international friendships were cemented and fruitful ideas exchanged. Such informal discussions between members of the Conference provided occasions for exchange of thoughts and experiences relating to electron microscope design, adjustment and technique, and relating to specimen preparation and interpretation of micrographs. To biologists such conversations were of especial value, in view of the revolution in cytological thought now under full headway, which receives much of its impetus through the medium of electron microscopy.

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## IS 'SUPER-REFINEMENT' LEGITIMATE IN X-RAY CRYSTAL ANALYSIS?

PROF. A. I. KITAJGORODSKIJ has directed attention to the importance of considering whether refinement beyond the normal Fourier stage of X-ray crystal structure analysis has any physical significance. As he points out, the legitimacy of the refinement process must be examined in the light of (a) the accuracy of the observed structure factors; and (b) the ratio of the number of arbitrary parameters (scaling factor, atomic co-ordinates, Debye

factors, atomic scattering factors, etc.) to the number of independent observations used in the analysis.

He is, of course, correct in pointing out that if the values of  $F_0$  are known with a precision  $\delta$ , then the minimization of  $R$  to a value smaller than about  $\delta$  is devoid of physical significance, and that a procedure of minimization in which the number of arbitrary parameters may be of the same order of magnitude as the number of reflexions is also meaningless. It

is also true that, in the past, crystallographers have not always been sufficiently careful in making sure of the accuracy of their intensity measurements, or in estimating or correcting for absorption, extinction and dispersion.

We do not, however, agree with his pessimism concerning the limit of accuracy attainable. He mentions crystals of 1 mm. thickness and points out that absorption and extinction can introduce large errors. Indeed they can. But in the majority of cases there is no need to use crystals of this large size. In recent investigations on anthracene and on the bicyclic dibenzaeridines<sup>2</sup>, made in University College, London, two-dimensional data were collected from crystals which were either cylindrical or spherical to a very close approximation. The large majority of reflexions (about 90 per cent of those possible using copper  $K\alpha$  radiation) were from crystals the maximum dimension of which was not more than 0.15 mm. A path difference of 10 per cent in such a small crystal of a purely organic compound would cause an absorption error of less than 0.5 per cent in the structure amplitudes. The remaining very weak high-order reflexions (about 10 per cent of the total) were obtained from crystals having a nearly uniform diameter of about 0.5 mm. Even if the structure factors so observed were in error by as much as 10 per cent, however, their inclusion in the Fourier series is better than their total neglect, or their inclusion only under the heading of 'less than' values.

An attempt to establish objectively the probable errors in our structure factor amplitudes, as determined from visual measurements of photographic diffraction intensities, has proceeded as follows. For any given crystal, the reliability factor  $R$  between a set of  $F_0$  and the same set read independently some weeks later was of the order of 1.5 per cent. Between sets of data from at least two different crystals,  $R$  was of the order of 3.0–3.5 per cent. This figure will undoubtedly vary from one substance to another and from one experimenter to another. Counter methods may be expected to give the most reliable results. In each structure analysis a measure of reproducibility or of internal consistency of the  $F_0$  should be made, in order to determine what limit of minimization of the index  $R = \sum |F_0| - |F_c| / \sum |F_0|$  may be expected.

Internal consistency, however, is not the only test of reliability of experimental results. Experience has shown us that whatever experimental data are used,

the refinement of the structure can proceed to an  $R$  factor only about 2 per cent higher than that of the data itself: if the accuracy of the observed structure factors is worse than 10 per cent and the number of unknown parameters in the structure is less than the number of  $F_0$ , then the refinement will not proceed to an  $R$  factor lower than about 12 per cent. In other words, we claim that the extent to which the structure can be refined is a measure of the reliability of the experimental observations, provided only that there is sufficient over-determination of parameters; and the higher the degree of over-determination the more certain is this test of reliability. Allowing for small random errors in the  $F_0$ , an allowance of three times as many observations as there are parameters to be determined is usually regarded as sufficient.

Prof. Kitajgorodskij is correct in pointing out that experimental accuracy is less easily obtained using crystals containing heavy atoms, but with care good results can be obtained<sup>3</sup>. Measurements on several crystals of different shapes, corrected for absorption and dispersion, can be compared. If extinction is present it shows itself in a variability of relative intensity for some of the strong and medium-strong intensities of low order. Recently<sup>4,5</sup>, methods of detecting extinction by the use of polarized incident radiation have been suggested, and these may lead to the application of a first-order correction.

Prof. Kitajgorodskij's major challenge, however, is in relation to the physical significance of any structure analysis that goes beyond a determination of the atomic co-ordinates. He claims that the maxima of an electron-density series, using only those amplitudes the signs of which are independent of the choice of  $f$  curves, give the co-ordinates of the 'centres' of the atoms, with a precision limited by the cut-off and the imprecise knowledge of the  $F_0$  values, and that *the structure analysis is then complete*.

We believe that he is wrong, and that this can be proved in several different ways.

(1) Experience has shown us that if the  $F_0$  values have an internal consistency of better than 5 per cent, but the anisotropies of the atomic vibrations and of atomic bonding are ignored, then refinement of the structure to better than about 15 per cent is impossible. This means that much valuable and reliable information is being wasted. A single least squares adjustment of thermal anisotropies, or a single refinement based on difference Fouriers, can in practice immediately improve the reliability factor.

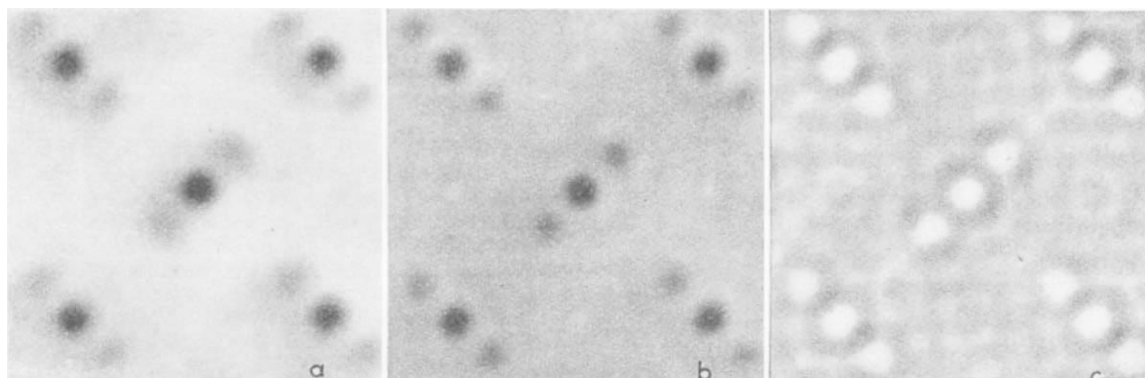


Fig. 1. (a) Optical synthesis of [001] projection of urea,  $\text{CO}(\text{NH}_2)_2$ , from observed diffraction data at 293° K. (b) Optical synthesis of structure factors calculated for a structure in which the atoms are not moving. (c) Difference synthesis between (a) and (b), showing the thermal vibrations and the false background detail due to 'termination of series' errors

(2) The electron-density map itself will often show directly that thermal vibrations and other anisotropic effects do exist and can be measured. In University College, London, chemistry undergraduates are given, as a practical exercise, the determination of the [001] projection of the structure of urea, using visually estimated  $\{hk0\}$  intensities and carrying out the electron-density projection by an optical synthesis of the  $F_0$  values. The resulting picture shows very clearly indeed that at room temperatures the molecules are oscillating through a small angle about the C=O bond (Fig. 1a). A similar optical synthesis using calculated  $F$  values corresponding to stationary atoms gives contours which are no longer anisotropic and diffuse, but the picture shows instead considerable false background detail due to series cut-off (Fig. 1b). Fig. 1c shows the thermal vibrations as revealed by a 'difference' synthesis.

(3) The existence of this molecular libration at room temperatures is confirmed by machine refinement of the electron-density series, which gives the vibration ellipsoids of the individual atoms<sup>6,7</sup>. It is also confirmed by an independent measurement of Debye factors for a number of crystal reflexions, using measurements of intensities made at several temperatures<sup>8</sup>. It is confirmed by an examination of the diffuse scattering patterns, which show strong streaks corresponding to the  $\langle 110 \rangle$  directions, which are directions of maximum atomic movements; and by the large thermal expansion in the  $\{hk0\}$  directions as compared with that along [001]. Finally, it is confirmed by the agreement, within limits of experimental error, between X-ray and neutron diffraction structure refinements (Worsham, jun., J. E., Levy, H. A., and Peterson, S. W., private communication).

No doubt the number of substances for which an accurate determination of atomic vibration amplitudes has been made is still very small indeed, and crystallographers are to blame for not having always realized the importance of exact experimental observations; but there seems no reason why, with the increasing use of computer facilities, we should not now get the utmost information out of our diffraction techniques.

One final word of warning is perhaps necessary. A Fourier technique that ignores the existence of thermal vibration effects may not even locate the 'centres' of the atoms accurately. If the atoms are moving on an arc—if, for example, they are part of a molecule that is oscillating about an axis or a fixed point—then the 'centres' of the atoms and the maxima of the electron-density series will not necessarily coincide<sup>9</sup>.

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<sup>1</sup> Kitajgorodskij, A. I., *Nature*, **179**, 410 (1957).

<sup>2</sup> Mason, R., *Nature*, **179**, 465 (1957).

<sup>3</sup> Evans, H. T., Tech. Report 58, Lab. for Insul. Research, M.I.T. (1953).

<sup>4</sup> Ramaseshan, S., and Ramchandran, G. N., *Acta Cryst.*, **6**, 364 (1953).

<sup>5</sup> Chandrasekhar, S., *Acta Cryst.*, **9**, 954 (1956).

<sup>6</sup> Vaughan, P., and Donohue, J., *Acta Cryst.*, **5**, 530 (1952).

<sup>7</sup> Grenville-Wells, H. J., *Acta Cryst.*, **9**, 709 (1956).

<sup>8</sup> Gilbert, R. E., and Lonsdale, K., *Acta Cryst.*, **9**, 697 (1956).

<sup>9</sup> Cox, E. G., Cruickshank, D. W. J., and Smith, J. A. S., *Nature*, **175**, 766 (1955).

IN a recent communication on this subject<sup>1</sup>, Prof. A. I. Kitajgorodskij concluded that "attempts at adapting X-ray structure analysis for the purpose of determining electron density or of determining the anisotropy of thermal vibrations are without foundation". If Prof. Kitajgorodskij had limited his criticism of current trends in X-ray analysis to the suggestion that some analyses have been refined further than the reliability or extent of the data warranted, he might possibly have found some supporting evidence. It is true that errors due to absorption and extinction can be important, and there are perhaps recent cases where they have been unjustifiably neglected. But as Lonsdale, Mason and Grenville-Wells point out above, experimental data of demonstrably high accuracy have been obtained in some cases, and there is no reason to doubt that they can be obtained in others. The sweeping assertion by Prof. Kitajgorodskij, which denies the possibility of finding anything more than the positions of atomic centres, even with the best experimental data, can be refuted on many grounds; but we think we need only point out (a) that the basic principles employed in 'super-refinement' have been accepted for a generation, and (b) that the validity of their application to increasingly complex structures can be tested by comparing the results with those obtained from entirely different experiments, namely, calorimetry, measurements of elastic constants, Raman spectroscopy, and nuclear magnetic resonance.

It must be emphasized that the principles employed in determining anisotropic thermal motion in complex crystals are not fundamentally different from those first used long ago in the determination of the isotropic motions and the electron densities in simple cubic crystals; an excellent account of the results is given by James<sup>2</sup>. As is well known, the amplitudes of isotropic thermal motion found by X-rays in these simple cases are in satisfactory agreement with those inferred, through the lattice vibration theory, from calorimetric and elastic measurements, while the atomic electron densities agree closely with those calculated by the self-consistent field method. As soon as we turn from cubic crystals to those of lower symmetry we find ample evidence, quite independent of X-ray diffraction, to show that in some cases at least the atoms are definitely not spherically symmetrical; we have only to recall, for example, the facts that the axial ratios of some of the metals which crystallize in the hexagonal close-packed arrangement differ from the ideal value of 1.63 by as much as 15 per cent, and that the ratio of their compressibilities parallel and perpendicular to the principal axis may be as high as 8. The inference that the thermal motions of the atoms should be treated as anisotropic is inescapable, and it is scarcely surprising to find that by introducing anisotropic thermal parameters we can bring the calculated X-ray intensities into better agreement with those observed experimentally than would otherwise be possible. The extension of these principles to more complex crystal structures is accompanied by the introduction of more parameters, but not more in proportion than the increase in the number of experimental observations, so that in general the parameters are still over-determined by a very wide margin. Provided that this safeguard remains, and the step from (say) potassium chloride to zinc is accepted, there is in the extended application of the principles to structures of greater and greater complexity no subsequent point at which it can be said