

(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^{6,7}. It is also confirmed by an independent measurement of Debye factors for a number of crystal reflexions, using measurements of intensities made at several temperatures⁸. 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 coincide⁹.

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March 5.

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In a recent communication on this subject¹, 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 James². 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

that 'illegitimate' methods have been introduced.

Actually Prof. Kitajgorodskij's view that the determination of the phases and the study of electron distributions and thermal motions are two separate operations, one legitimate and the other not, is an erroneous over-simplification. His references to the 'signs' of the structure factors show that his considerations are restricted to centrosymmetrical crystals. In the general case, the structure factors are complex and their phases are infinitely variable parameters, which are to be dealt with, in the one operation of determining the structure, along with the other parameters of the problem. Even the study of a centrosymmetrical cubic crystal (caesium antimonide)³ can show how illusory any general attempt to separate phase determination from thermal parameter determination must be.

In conclusion, it is relevant to mention that some anisotropic thermal parameters are more liable than others to be affected by absorption and extinction errors. Roughly speaking, neglect or imperfect correction for these errors causes a systematic error in the mean Debye parameter B of the atoms in the isotropic case, or systematic errors in the mean atomic vibration tensor \mathbf{U} in the anisotropic case⁴. In crystals with sufficiently rigid molecules, the atomic vibration tensors \mathbf{U}^* can be analysed⁵ in terms of two tensors, \mathbf{T} , giving the anisotropic translational vibrations of the mass centre of the molecule, and

ω , giving the anisotropic angular vibrations of the molecule about axes through its centre. Systematic errors in the mean \mathbf{U} would affect \mathbf{T} , but it is rather difficult to see how they could systematically affect ω . If Prof. Kitajgorodskij's conclusions were true, one would not expect the calculated values of \mathbf{T} and ω in general to have any obvious connexion with physical realities; nor would it be surprising to find cases where the latent roots of ω were negative (corresponding to the centre of a molecule having a greater angular vibration than its periphery). In fact⁶ the values found for \mathbf{T} and ω are usually very reasonable, and they imply lattice frequencies⁷ which check closely with the spectroscopic and thermodynamic data⁸.

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INFECTIVITY OF RIBONUCLEIC ACID FROM EHRLICH ASCITES TUMOUR CELLS INFECTED WITH MENGO ENCEPHALITIS

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GIERER and Schramm have described a method for the isolation of ribonucleic acid from tobacco mosaic virus and have presented evidence to show that nucleic acid so prepared is itself infectious¹. Two of us have reported some of the chemical and physical properties of ribonucleic acid isolated from Ehrlich ascites carcinoma cells by their technique². The present communication presents evidence that ribonucleic acid isolated from Ehrlich ascites tumour cells infected with Mengo encephalitis virus is infectious, and that a ribonucleic acid component, rather than residual intact virus particles, is responsible for this activity.

The Ehrlich ascites carcinoma was grown in Swiss albino mice. On the seventh day of tumour growth, 0.5 ml. of a 10⁻⁴ dilution of Mengo virus-infected mouse brain homogenate was injected intraperitoneally. The multiplication of this virus in, and its powerful oncolytic action on, cells of the Ehrlich ascites tumour have been described by Koprowska and Koprowski³.

Ascitic fluids were collected by aspiration from the peritoneal cavities of tumour-bearing mice 48–72 hr. after virus infection. Cells were sedimented by low-speed centrifugation, washed several times in saline,

and frozen rapidly in a mortar set in an ethanol–'dry ice' bath. The frozen cells were ground by hand to a fine powder and were homogenized in a Waring blender with 0.14 *M* sodium chloride–0.01 *M* sodium citrate, pH 7.0, to give a 10 per cent (w/v) suspension. The suspension was centrifuged at 3,000 rev./min. for 20 min., and the supernatant decanted through a fluted Whatman No. 1 filter paper. Extracts prepared in this manner were considered to be 10⁻¹ dilutions of virus, and are here called Mengo encephalitis virus preparations.

Ribonucleic acid was separated from such virus preparations by the method of Gierer and Schramm¹. In brief, this consisted of extracting the virus preparation three times by vigorous shaking with an equal volume of water-saturated phenol. After each extraction, the aqueous phase which contained the ribonucleic acid was separated by centrifugation. The aqueous phase was freed of phenol by several ether extractions, and the ether was blown off by bubbling nitrogen gas through the solution. All manipulations were carried out at 0–4° C. Ribonucleic acid solutions so prepared were designated as 10⁻¹ dilutions for convenience in comparing the relative infectivities of Mengo virus and ribonucleic