PHYSICAL APPROACH TO THE STUDY OF BIOLOGICAL SYSTEMS

ONE of the many valuable features of the Institute of Physics Convention held in Buxton during May 19–21 was the joint meeting of the X-ray Analysis and Electron Microscopy Groups of the Institute for the purpose of exploring the physical approach to the study of biological systems. The discussion was opened by Prof. J. T. Randall (King's College, London), who was followed by Dr. I. M. Dawson (National Institute for Medical Research, Hampstead), Dr. R. Reed (University of Leeds), Dr. M. Perutz (University of Cambridge) and Mrs. D. M. Hodgkin (University Museum, Oxford).

It is not uncommon for scientific workers entering some new field of work such as biophysics to ignore or neglect much work and endeavour of great importance carried out by men of other disciplines than their own. Biologists have for hundreds of years been among the most conspicuous users of microscopes. The title of the symposium reported in these notes is, however, justified by the notable advances recently made by physicists in the development of new or improved instruments and methods of approach. X-ray diffraction, phase-contrast microscopy, reflecting microscopes and, not least, the electron microscope, have all opened up wide—and often quite new—fields of research in biology as well as in other subjects.

In his introduction to the symposium, Prof. J. T. Randall chose to speak of certain problems not specifically dealt with by other contributors, emphasizing how useful is the manifold approach to complex problems, particularly in the biophysical field, where the external distinction of form, and perhaps of crystallinity, merge into the less definite features of micro-morphology, cytology, and its biochemical interpretations. As an example, Prof. Randall gave the work of M. B. McEwan and D. L. Mould on the properties of bentonite gels in relation to long-distance forces. In order to understand these systems, it has been necessary not only to devise methods of measuring the rigidity, but also to study birefringence, X-ray and optical scattering, in addition to the measurement of particle-size in the electron microscope.

The application of X-ray diffraction methods to liquids, solutions and colloid systems has a history[1] almost as long as that of X-ray crystallography proper, and the recent work of Riley[2] and Riley and Herbert[3] on solutions of hæmoglobin and egg albumen shows that valuable if limited information about molecular size, shape and aggregation may be obtained by the use of the low-angle scattering technique earlier applied with such success by Bernal and Fankuchen[4] to tobacco mosaic virus paracrystals. There is still too great a tendency in work on solutions to avoid the tedious but sometimes necessary procedure of deducing the radial distribution function of scattering matter by the method of Fourier inversion. Oster[5] has recently pointed out the formal similarities of the theories of X-ray and light scattering of solutions, and we may expect an increasing use of the two methods in the study of size, shape and interaction of large molecules.

There has also been increasing interest in the application of other optical methods to the study of biological material. This is evident in the ultra-violet[6] and infra-red[7] fields, where the advantages of the reflecting microscope[8,9] are bound to be exploited, especially with living material. Dichroism, microscopy and absorption at low temperatures[10,11] are also aspects of biophysical study likely to develop.

The discussion on the use of the electron microscope in the study of biological material revealed that a very wide range of problems is being studied in Great Britain. The paper by Dr. R. Reed, of Leeds, reviewed work by Wyckoff, Cosslett and others on crystalline viruses and the relation of this work to that of the X-ray crystallographers. The limit of resolution in the electron microscope has so far restricted such studies to the viruses of higher molecular weight.

Dr. Reed also referred to the relationship between globular and fibrous proteins, a relationship which has been brought very much to the forefront by recent studies in Great Britain (at Leeds) and in the United States (Wyckoff, Porter and Hall). It is becoming clear that many of (possibly all) the fibrous proteins can exist in a unit, corpuscular form which has the property of forming long fibres by linear aggregation. Actin, tropomyosin, fibrinogen-fibrin, and certain collagenous fibrils should be mentioned in this connexion. This important question was later discussed by Perutz.

Dr. Dawson referred particularly to the study of individual virus particles and the wide variation in structure which may exist; the larger viruses, such as vaccinia, may be more akin to bacteria and the smaller ones to protein molecules. Such generalizations, however, may be swept away by our growing knowledge of the smaller units. The importance of enzymic digestion in the elucidation of virus structure was also brought out in Dr. Dawson's paper, and mention was made of the use of trypsin, pepsin, desoxyribonuclease, collagenase and lecithinase.

The work of Elford and Dawson on the multiplication of virus particles within the host cell was also discussed. Hirst's technique for the separation of virus particles of the influenza group by means of adsorption and elution from the surface of red blood cells has been adapted to the electron microscope by the use of laked cells in order to achieve a sufficiently thin subject for examination. The examination of a strain of influenza virus such as *PR*8 at various stages of the development cycle has given suggestive evidence concerning multiplication by binary fission.

Prof. Randall referred to work on tissue culture cells, nuclear membranes and spermatozoa, and stressed that, as the electron microscopist advances from the study of single particles to an organised or differentiated biological structure, the need for specific 'electron' stains comparable with the well-known stains used in visible microscopy is becoming an urgent problem, without which electron microscopy cannot hope to share in the development of cytochemistry. While the technique of tissue culture provides thin, flat cells for examination, it is also clear that more reliable sectioning techniques are required.

Dr. M. Perutz gave an appropriate and clear survey of recent work on the structure of proteins as one of the most interesting meeting points of X-ray analysis and electron microscopy. After some reference to the X-ray work of Astbury on protein fibres produced by the living cell, and to the conclusion that these fibres consist of bundles of polypeptide chains running parallel to the fibre direction, Dr. Perutz went on to discuss the evidence of electron microscopy. Contrary to the older ideas of micelles, there always

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seems to be at least one level of organisation between the polypeptide chain and the macroscopic fibre. This level consists of microfibrils the thickness of which varies between 100 A. and 1,000 A. in different proteins ; and the microfibrils in turn often appear to consist of assemblies of globular particles ; sometimes a simple string of beads ; sometimes a more complex pattern as in the paramyosin of clam muscle. The investigation of clam muscle is a striking example of the combined approach of X-ray analysis and electron microscopy. The X-ray pictures of Bear[12] show the typical α-keratin pattern in addition to a low-angle pattern which is resolved into a series of distinct spots. The low-angle pattern can be interpreted in terms of hexagonal array of globular particles of 150 A. diameter, which corresponds exactly with the electron microscope pictures of Hall, Jakus and Schmitt[13]. Another protein, tropomyosin, can undergo a reversible transformation from fibrous to what may prove to be a corpuscular state. The work of Perutz and Kendrew on hæmoglobin and myoglobin[14], both typical corpuscular proteins which have no tendency towards fibre formation in the native state, shows these proteins to consist of bundles of polypeptide chains. It appears that the chains are folded in the α-keratin configuration. Mrs. Hodgkin[15] has recently found evidence of such chain structures in the small peptide gramicidin. Dr. Perutz stressed the importance of further knowledge of the α-keratin structure as basic to the future study of hæmoglobin and myoglobin ; this knowledge is not likely to come by model-building alone ; more experimental data are required. The recent studies by Elliot, Ambrose and Temple[16] with polarized infra-red radiation on proteins of the α-keratin type have revealed a striking dichroism. Absorption is much stronger (in the neighbourhood of 3 μ) parallel to the fibre direction than at right angles to it. This indicates that the CO—NH bonds are oriented preferentially and parallel to the fibre axis. This observation confirms the old contention that folds in the α-keratin chain must be held together by hydrogen bonds located within each chain.

The use of the reflecting microscope in such studies receives further emphasis from the work of Barer, Jope and Perutz on the ultra-violet absorption of single hæmoglobin and myoglobin crystals. The pronounced dichroic effect in the absorption of the hæm group at 400 mμ shows that this ring is orientated with its plane normal to the *a*-axis of the crystal. The second absorption band shows a maximum dichroic effect at 290 mμ, a region associated with the indol group of tryptophan. Like hæm, this ring system would be expected to show absorption only when the electric vector is parallel to the plane of the ring. The dichroic effect thus indicates that the plane of the indol group is normal to the *a*-axis. X-ray data show the polypeptide chains to be parallel to *a*. Thus, the planes of both hæm and indol rings are normal to the polypeptide chain direction. In tobacco mosaic virus the indol rings are normal to the length of the virus particle.

Dr. Hodgkin stressed the importance of obtaining new information about the region of size to which both X-ray diffraction and the method of electron microscopy might apply ; and particularly, the region just below the limit of size where electron micrographs at present show clear detail, say 50 A. or so. X-ray data indicate structure within particles such as the bushy stunt or turnip yellow virus which might be of this order of magnitude. Dr. Hodgkin also discussed the interrelation between information derived from both methods as applied to tobacco necrosis protein.

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[1] See, for example, Randall, J. T., "Diffraction of X-rays by Amorphous Solids and Liquids" (London: Chapman and Hall, 1934).
[2] Riley, D. P., *Brit. Sci. News* (in the press).
[3] Riley, D. P., and Herbert, D., *Biochim. et Biophys. Acta* (in the press).
[4] Bernal and Fankuchen, *J. Gen. Physiol.*, **25**, 111 (1941).
[5] Oster, *Rec. Trav. Chim. Pays-Bas* (in the press).
[6] See, for example, Caspersson in Society of Experimental Biology, Nucleic Acid Symposium (Cambridge, 1947).
[7] See *Proc. Roy. Soc.*, B, **136**, XIV (1949).
[8] Burch, *Proc. Phys. Soc. Lond.*, **59**, 41 (1947).
[9] Seeds and Wilkins, *Nature*, **164**, 228 (1949).
[10] Brown and Randall, *Nature*, **163**, 209 (1949).
[11] Loofbourow, Sinsheimer and Scott, *Science*, **107**, 302 (1948).
[12] Bear, *J. Amer. Chem. Soc.*, **66**, 2043 (1944).
[13] Hall, Jakus and Schmitt, *J. App. Phys.*, **8**, 459 (1945).
[14] Perutz and Kendrew in Barcroft Memorial Volume "Hæmoglobin", 161 (Butterworth, 1949).
[15] Hodgkin and Schmidt, reported in *J. Sci. Instr.*, **26**, 283 (1949).
[16] Elliot, Ambrose and Temple, *Nature*, **163**, 859 (1949). See also Astbury, *Nature*, **164**, 439 (1949) and Sutherland and Darmon, *Nature*, **164**, 440 (1949).

PREPARATION AND INSECTICIDAL ACTION OF *BIS* (*BIS*-DIMETHYLAMINO)-PHOSPHONOUS ANHYDRIDE

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IT has been reported by Schrader[1,2] and other German workers that certain organo-phosphorous compounds have strong insecticidal properties. They act as contact insecticides, but also, in a less familiar manner, as 'systemic' insecticides, that is, they are absorbed by the roots or the leaves of a plant and render it toxic. The effect may be very persistent and this mode of action would be of considerable economic interest were it not for the fact that, with compounds tested to date, the treated plants are also toxic to mammals.

In order that biological tests may be definitive, it is necessary to work with pure materials. This is especially so with the synthetic organo-phosphorous compounds, which differ widely in insecticidal activity. The *bis* (*bis*-dimethylamino)-phosphonous anhydride used in the present investigation has been prepared with great care, and the steady boiling point and satisfactory analysis suggest that the material was pure.

Preparation. Purified starting materials were employed and the intermediate compounds were all fractionated carefully one or more times through a 24-in. column packed with glass helices and with a variable take-off stillhead. The method of synthesis used was based on that outlined by Schrader[2] and was briefly as follows :

Methylaminehydrochloride and excess phosphorus oxychloride were refluxed for 20 hours and fractionated to give dimethylamino-dichlorophosphine oxide (I), NMe_2POCl_2 (b.p. 88°/18 mm. ; 94 per cent yield). Treatment of I (1 mol.) with methylamine (2 mol.) in ether at room temperature yielded *bis* (dimethylamino)-chlorophosphine oxide (II), $(NMe_2)_2POCl$

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