CIBA FOUNDATION SYMPOSIUM

ON

THE NATURE OF VIRUSES

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Editors for the Ciba Foundation

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and

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PREFACE

THE suggestion that one of the Ciba Foundation's small, informal, international conferences should provide an opportunity for discussion of some fundamental aspects of virology originated in Cape Town, in conversations between Professor M. van den Ende and Professor F. G. Young. The latter, as a member of the Council of the Foundation, pursued the matter in detail on his return to England, and the Director readily took up the proposal.

The symposium was arranged for March, 1955, under the title of "The Biophysics and Biochemistry of Viruses". Sir Charles Harington, Director of the National Institute for Medical Research, consented to act as Chairman, and also gave invaluable advice on membership and the construction of the programme. Perhaps even more than on previous occasions, the Director regretted his inability to include in so small a group many of the outstanding contributors in this field of research. Those who could be invited and who honoured us with their presence and contributions were as usual most helpful and co-operative, both in the discussions and in the preparation of this subsequent publication. The Director hopes to be able to invite other virologists on appropriate occasions, and in the meanwhile offers them and other interested workers such participation in this symposium as this volume can give them.

A few explanatory words about the Ciba Foundation may be useful here, though this is the 32nd book containing proceedings of our conferences to be published, and one or more of our other activities may have come to the reader's attention.

The Ciba Foundation is an international centre, established as an educational and scientific charity under the laws of England. It owes its inception and support to its Founder, CIBA Ltd. of Switzerland, but is administered independently and exclusively by its distinguished British Trustees.

The Foundation provides accommodation for scientific workers who visit London from abroad, organizes and holds international conferences, conducts (in conjunction with the Institut National d'Hygiène) a postgraduate medical exchange scheme between England and France, arranges informal meetings for discussions, awards two annual lectureships, has initiated a scheme to encourage basic research relevant to the problems of ageing, assists international congresses and scientific societies, is building up a library service in special fields, and generally endeavours to give aid in all matters that may promote international co-operation in scientific research.

List of those participating in or attending the Symposium on "The Biophysics and Biochemistry of Viruses", 26th–28th March, 1956

G. L. Ada	The Walter and Eliza Hall Institute of Medical, Research, Melbourne
C. H. Andrewes	The National Institute for Medical Research, Mill Hill, London
F. B. Bang	Dept. of Pathobiology, Johns Hopkins School of Hygiene, Baltimore, and The National Institute for Medical Research, Mill Hill, London
F. C. Bawden	Rothamsted Experimental Station, Harpenden, Herts
G. L. Brown	Biophysics Research Unit, King's College, London
Sir Macfarlane Burnet	The Walter and Eliza Hall Institute of Medical Research, Melbourne
D. Caspar	The Josiah Williard Gibbs Research Laboratory, Yale University, and Cavendish Laboratory, University of Cambridge
A. H. Coons	Dept. of Bacteriology and Immunology, Harvard Medical School, Boston
F. H. C. Crick	The Medical Research Council for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge
A. W. Downie	Dept. of Bacteriology, University of Liverpool
R. Dulbecco	Division of Biology, California Institute of Technology, Pasadena
M. van den Ende	C.S.I.R. and U.C.T. Virus Research Unit, Dept. of Pathology, University of Cape Town
Rosalind E. Franklin	Birkbeck College Crystallography Laboratory, University of London
I. A. Galloway	Research Institute, Pirbright, Surrey
S. Gard	Dept. of Virus Research, Karolinska Institutet Medical School, Stockholm
Sir Charles Harington	The National Institute for Medical Research, Mill Hill, London
J. I. Harris	Dept. of Biochemistry, University of Cambridge
G. K. Hirst	Division of Infectious Diseases, The Public Health Institute of the City of New York, Inc.

L. HOYLE	Public Health Laboratory, General Hospital, Northampton
A. ISAACS	The National Institute for Medical Research, Mill Hill, London.
A. KLUG	Birkbeck College Crystallography Laboratory, University of London
C. A. KNIGHT	Virus Laboratory, University of California, Berkeley
F. KRADOLFER	Biology Division, CIBA Ltd., Basle
A. LWOFF	Institut Pasteur, Paris
R. MARKHAM	A.R.C. Plant Virus Research Unit, Molteno Institute, Cambridge
N. W. PIRIE	Rothamsted Experimental Station, Harpenden, Herts
M. R. J. SALTON	Dept. of Bacteriology, University of Manchester
F. K. SANDERS	M.R.C. Virus Research Group, London School of Hygiene and Tropical Medicine
W. SCHÄFER	Dept. of Virology, Max-Planck Institut, Tübingen
WILSON SMITH	Dept. of Bacteriology, University College Hospital Medical School, London
E. T. C. SPOONER	Dept. of Bacteriology, London School of Hygiene and Tropical Medicine
M. G. P. STOKER	Dept. of Pathology, University of Cambridge
J. D. WATSON	Biological Laboratories, Harvard University, and Cavendish Laboratory, University of Cambridge
R. C. WILLIAMS	Virus Laboratory, University of California, Berkeley

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Chairman: SIR CHARLES HARINGTON

CHAIRMAN'S OPENING REMARKS

SIR CHARLES HARINGTON

I FIND my task in opening this symposium a somewhat embarrassing one, since I am the only member of the group who has made no scientific contribution whatever to the subject under discussion; in these circumstances I can speak only as a biochemist who has long been interested in the progress of virus research and who, because of the nature of his job, has attempted to maintain a nodding acquaintance with developments in the field. In this capacity I would like to say first that the most satisfactory feature of the present occasion is that the time has come when it is worth while to hold a symposium within our terms of reference; secondly, may I say how encouraging it is to see gathered here today so many of those to whose efforts the important advances have been due.

It happens that the Institute which I have the honour to direct has been a home of virus research for many years. The history of the work on viruses that has been carried on there goes back indeed to the time when virology had not yet emerged as an independent subject of research and when progress was still inhibited by failure to realize that the elementary biological facts concerning the behaviour of viruses could only be revealed by studies of the infection in the host. We ourselves are proud to remember, as two outstanding events in the scientific history of our Institute, that studies of this kind carried out there led to the major discoveries of the virus of dog distemper and of that of influenza, thus opening up a very wide field of work.

Intrinsically important as these biological discoveries were, however, they served themselves to emphasize that for a comprehensive advance in knowledge of the properties of viruses the help of other branches of science would have to be

called in. One of the chief preoccupations of the early workers on viruses was the question of the particle size of the agents that they were studying, and the first real contributions to this problem were provided by the work of Barnard in the development of ultraviolet microscopy and by that of Elford on the preparation and use of filtration membranes of graded porosity. It is indeed remarkable that by this simple method Elford was able to make estimates of particle size so closely in accordance with the values that have been obtained in later years by the use of more complex and accurate physical procedures.

The work of Barnard and Elford represents, however, no more than the first elementary attempt to apply the methods of physics to the study of viruses and it leaves untouched the question of their chemistry. The possibility of anything like exact study of the chemistry and physics of viruses had to await the great achievement of Stanley when, in 1986, he succeeded in crystallizing tobacco mosaic virus; this work, with that of Bawden and Pirie and others, immediately made the chemical and physical study of plant viruses an attractive proposition in so far as the material to be studied satisfied at least one criterion of purity and homogeneity. Concurrently the advances in biological methods of cultivation of animal viruses, arising from the egg culture methods of Goodpasture and Burnet and the more recent tissue culture methods developed by Enders and others, have made it possible to obtain these viruses also in what appears to be a homogeneous state, and therefore in a suitable condition for the physicist and chemist to work on them.

What use has been made of these opportunities? Surely a very full one and one that is ever increasing in vigour as we shall learn in the course of this symposium. On the physical side, knowledge of the size and morphology of virus particles has been revolutionized by the skilful application of the high-speed centrifuge and of the electron microscope, the capacities of which have been and are being stretched to the limit in the effort to reveal more details of structure. As for what one

might call the elementary chemistry of viruses, the crystallization of plant viruses has become a commonplace; it is indeed interesting to compare the matter of fact reception of the recent news of the extension of crystallization to the field of animal viruses in the work on poliomyelitis with the intellectual shock that was administered by Stanley's discovery twenty years ago.

Even more impressive is the detailed biochemical analysis of viruses that has been carried out during the last few years and is being so actively pursued today. We have travelled a long way from the mysterious filtrable infective particle of little more than thirty years ago to the present stage when we can envisage a typical virus particle as a structure made up of nucleic acid with a coat of protein. Furthermore, we can distinguish between the two parts of the virus structure, regarding the nucleic acid as the genetic material, whilst the protein coat determines antigenic specificity and provides the mechanisms for attachment of the virus to the host cell and penetration of the genetic material into the cell; presumably also the protein coat carries the specific enzymic properties associated with viruses such as influenza. Now we have even the evidence that the protein and nucleic acid portions of certain plant viruses can be dissociated and later recombined to form a reconstituted infective particle, and that, in some cases at least, a partial breakdown of the protein portion is consistent with retention of infectivity.

Clearly discoveries of this sort are providing the basis for an understanding of the host-virus relationship and of the process of virus multiplication. These are not only phenomena of the greatest intrinsic biological interest but they have implications extending far beyond the field of virus research. For virus multiplication is after all a special case of protein biosynthesis, and there is no doubt that this general problem will be illuminated by the work that is being done on the structure of viruses and on their chemical composition. In saying this I have in mind such theories of virus protein structure as that developed by Crick on the basis of

the study of bushy stunt virus and also the discovery of the unusual base hydroxymethylcytosine in phage nucleic acid, which in Cohen's view provides a biological trap mechanism for the diversion of nucleic acid synthesis from host to virus. Finally, we must remember that the practical objective of all virus research is the discovery of methods of controlling virus multiplication; so far as chemotherapeutic control is concerned there is no direction from which the solution of this problem is more likely to come than the biochemical study of viruses, and the remarkable experiments that have been done on the incorporation of abnormal bases into virus nucleic acid may well point the way that we should follow.

We seem thus to have reached a point at which biochemical and biophysical studies of viruses have really come into their own and offer the greatest prospects of advance. It was this thought that encouraged those who were responsible for arranging this symposium, and they will have their reward if the exchange of views that is to take place here during the next three days does something to accelerate still further the progress that is already so encouraging.

VIRUS STRUCTURE: GENERAL PRINCIPLES

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Introduction

IN this article we shall discuss some general ideas about the structure of viruses. This is a hazardous undertaking. We know of no principles so compelling that we can be certain that they must be true; or, more correctly, those that must be true—the rules for inter-atomic distances, for instance—do not lead directly to any interesting conclusions. However, there are certain ideas suggested by experience in related fields (such as the study of protein crystals) which we might well expect to apply to viruses, or at any rate to small viruses. Moreover we can make some use of that powerful but dangerous weapon, the principle of simplicity.

Our ideas fall into two groups. There is good evidence in the case of three plant viruses, and indirect evidence for certain animal viruses, that the protein component of a virus is made up of sub-units. Our first set of ideas concerns the question: why does a virus have protein sub-units? We have not previously published this argument. Our second deals with the problem: if there are sub-units, how are they arranged? This we have recently put forward elsewhere, so that we shall only deal with it briefly. This paper should therefore be read in conjunction with our previous one (Crick and Watson, 1956).

We shall restrict our discussion in the first place to those small viruses which contain only protein and ribonucleic acid (RNA): that is, the majority of known plant viruses, and

* On leave from the Biology Department, Harvard University, and supported by a grant from the National Science Foundation (U.S.A.).

certain animal viruses such as poliomyelitis and the various encephalitic viruses.

The reason for protein sub-units

Our basic argument is that the protein component of a virus is unlikely to be either one large molecule or, alternatively, an assembly of small molecules, each of which is quite different from all the others. More precisely, we mean by "different" that the sequence of amino acids in any two such small molecules is quite unrelated.

Our first assumption is that an essential requirement for a virus of this type is that it should consist of a packet of RNA protected by a coat of protein. It is found experimentally that the molecular weight of the RNA is of the order of, say, 2×10^6. Imagine that this amount of RNA is folded as compactly as possible, so that it forms a rather dense sphere. Such a sphere could hardly be less than 150 Å diameter, and is more likely to be nearer 200 Å. We next surround this with a layer of protein, which we shall assume is more or less continuous. There must be a minimum thickness for such a layer; 1 Å, for example, would be impossibly small. A more reasonable minimum value would be 10 Å. Actually no protein crystal is known with a unit cell dimension of less than 24 Å, so that perhaps 20 Å would be a more realistic lower limit. This would require a volume of protein of about 10^7 Å^3, or a molecular weight near 7 million. The details of the calculation are unimportant; the point is that we require a large amount of protein. Notice that the ratio of protein to RNA *increases* as we make the virus smaller; that is, if we had considered a smaller amount of RNA we should not reduce the amount of protein required by very much. We can only have a much smaller *proportion* of protein if the virus is considerably bigger.

The model we have described must not be taken as a detailed model of a virus. It is used purely for illustration. If we follow through the argument for a rod-shaped virus of small diameter we reach a similar conclusion.

Thus, if our assumption that a small virus has to have a reasonably continuous protein coat is correct, we can conclude that a relatively large amount of protein will be required for it. Whatever the reason, the experimental evidence shows clearly that a considerable amount of protein is always present. In Table I we have set out the figures for all the

Table I

AMINO ACID AND NUCLEOTIDE RESIDUES IN RNA VIRUSES

	Molecular Weight	% *RNA*	% *Protein*	*Nucleotides per Particle*	*Amino Acids per Particle*
Tobacco mosaic virus	40×10^6	6	94	7,300	340,000
Potato virus X	$\simeq 30 \times 10^6$	6	94	5,400	260,000
Potato virus Y	75×10^6	5	95	11,300	650,000
Bushy stunt virus	9×10^6	16	84	4,400	69,000
Turnip yellow mosaic virus	5×10^6	40	60	6,000	27,000
Southern bean virus	6×10^6	21	79	3,800	43,000
Tobacco ringspot virus	$\simeq 6 \times 10^6$	40	60	7,300	33,000
Tobacco necrosis virus	$\simeq 6 \times 10^6$	18	82	3,300	45,000
Poliomyelitis	10×10^6	24	76	7,300	69,000
Influenza*	100×10^6	2	75	6,000	680,000
Fowl plague*	100×10^6	2	75	6,000	680,000

The figures in this Table are only approximate

* These viruses may contain material from their host cell

small viruses for which data are available. It can be seen that in every case the *total* number of amino acids always greatly exceeds the *total* number of nucleotides.

Our next assumption is more difficult to justify. It really falls into three parts. We assume (*a*) that the amino acid sequence of the protein component of the progeny is determined wholly, or at least to a large extent, by the infecting virus; (*b*) that this amino acid sequence is determined by the molecular structure of the RNA of the infecting virus, and not at all by its protein component; (*c*) that the "coding" implied in (*b*) is relatively simple.

Of course none of these assumptions is new, though we believe that our argument as a whole is original.