Ciba Foundation Symposium

CELLULAR BIOLOGY OF MYXOVIRUS INFECTIONS

Edited by G. E. W. WOLSTENHOLME,

and

JULIE KNIGHT,

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The Ciba Foundation

for the Promotion of International Co-operation in Medical and Chemical Research
41 PORTLAND PLACE, LONDON, W.1

Preface

THIS symposium arose out of the suggestion by Professor A. P. Waterson that the Ciba Foundation's earlier conference in 1956 on "The Nature of Viruses" could now usefully be followed up by one concerned exclusively with the Myxoviruses, which are now in the forefront of viral research. We are particularly indebted to Professor Waterson for information on which the scope and membership of the meeting were largely based, and for his unexpected and most welcome help in the preparation of papers and discussions for publication in this volume. Our thanks are also very warmly due to Sir Christopher Andrewes, who guided the meeting with unobtrusive firmness and with every encouragement to its members to give the most to—and get the most out of—the discussions.

The Editors hope that the proceedings give a fair presentation of knowledge of these viruses in 1964, and that the book will be informative and stimulating to workers in this field.

The Editors would also like to record the participants' and the Foundation's regret that serious illness prevented the attendance of Dr. A. Isaacs, whose own investigations have contributed so much, directly and indirectly, to virological research throughout the world.

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CHAIRMAN'S OPENING REMARKS

SIR CHRISTOPHER ANDREWES

IN discussing the cellular biology of myxovirus infections we are in a way, I think, going back to medical school, because we are going to discuss anatomy and physiology. The difficulty with these small viruses is that in the fields of both their anatomy and their physiology we get so mixed up in chemistry that it is hard to know where one begins and the other finishes. The myxoviruses are nevertheless peculiarly suitable viruses for this discussion; they are not so small as poliovirus and other picornaviruses—so small that you cannot really find out very much about them; and they are not so large and complex as the pox viruses, where again it is difficult to find out exactly where you are; myxoviruses fall into a very convenient intermediate position, and more work has probably been done on them than on any other viruses. When Wilson Smith and I first got influenza virus going in ferrets, and thus first became involved with the myxoviruses, I remember naïvely thinking what fun it would be if a lot of people started to work on this virus and if there came to be a literature on this subject as vast as that which there then was on yellow fever. I little thought that thirty years later the American Institute of Biological Sciences would be sending out every quarter an abstract of all the current papers on influenza amounting to thirty pages in all; it would have appalled me at that time.

What I hope will come out of this discussion is not only an enlargement of our knowledge about myxoviruses; I hope that what we learn about them will also bring to light some general principles which will help us to understand the viruses which are not quite so easy to study. To give one example of the sort of way

in which this discussion may help virology in general, I will ask your forgiveness for demanding a little help on a problem concerning me personally. As some of you probably know, Dr. André Lwoff, aided and abetted by Dr. Horne, has been concerned in scrapping the existing Sub-committee for Virus Nomenclature and arranging for a provisional committee which is to try to do something about nomenclature until the next congress, in Moscow, makes an "honest woman" of it and reconstitutes it as a Virus Nomenclature Committee, subordinate only to the International Association of Microbiological Societies and no longer subservient to the Bacteriological Committee. In other words, there is to be a recognition that virology is a science on its own and of equal importance with bacteriology, and one thing the provisional committee is to do is to consider whether it is advisable to have a separate code of nomenclature for viruses.

As a preliminary to that we have got to clarify our ideas about the classification of viruses, and this is where I hope that we may be helped by this meeting. For instance, I should like to know: what is a myxovirus? I feel rather like the learned judge who asks a silly question in order to help to clarify the position and in so doing deliberately parades his own abysmal ignorance; but I differ from the learned judge in that my abysmal ignorance is not all simulated! At the same time I do think it is important from the point of view of general principles to find out how we are going to decide what a myxovirus is. It may seem rather nonsensical that I should be asking this question, since I was one of the authors of a paper which defined a myxovirus some years ago (Andrewes, Bang and Burnet, 1955), but we have learnt a great deal about myxoviruses since then and it is quite possible that we may want to revise our definition.

The basic point that has to be settled is whether morphology and chemical composition should be the fundamental characters on which we are going to classify viruses. If they are, it is probably going to be necessary to admit that dog distemper, measles and

rinderpest are myxoviruses, also the respiratory syncytial virus, and possibly others. Going a little further afield, it may be necessary to consider whether the fowl leukaemia viruses, the mouse leukaemia viruses and rabies are going to come into the same category. That is a matter to which I don't altogether know the answer and I hope that in the course of the meeting we shall get a little information which will make it easier to form an opinion.

One difficulty about making morphology and chemical composition our fundamental criteria for classifying viruses is that we have then logically to admit, as Lwoff would like to do, that the plant and animal viruses are all mixed up together (Lwoff, Horne and Tournier, 1962). There are some plant viruses morphologically related to animal viruses, and as I have been concerned for a long while in emphasizing the importance of fundamental characters against comparatively unimportant ones like clinical aspects and pathology, I feel very bothered by this, because I dislike the idea of mixing up animal and plant viruses and yet I cannot see any good logical reason why you should not.

Another matter concerning morphology bothers me and I hope that I shall be informed about this also. I received recently two reprints from Cape Town, one of them concerning the morphology of African horse sickness and the other about a Tern virus, which is essentially fowl plague. Both these descriptions refer to radiating spikes on the surface of these organisms, and in the case of the African horse sickness, these spikes are arranged symmetrically and definitely fall into the category of what are called capsomeres (Polson and Deeks, 1963), but in the case of the Tern virus they are not arranged in a symmetrical manner (Becker, 1963). What I want to discover is this: what is the fundamental difference between a capsomere and these radiating spikes on the surface of a myxovirus? I know that one difference is that they lack a regular arrangement, and that in the case of the myxovirus they are mixed up with lipids and other things, but is there an absolutely fundamental difference? Another

question arises in regard to the helical nucleoprotein in the middle of a myxovirus: is that fundamentally different from the nucleic acid in the middle of a polio virus? Do we know there is no protein inside poliovirus? Is this a difference of degree or is it as fundamentally different as it has generally been supposed to be?

I hope this mild digression has not taken us too far from the main subject of our discussion, because obviously the anatomy of these viruses is only one aspect of them. Their method of replication, the state of their surfaces, their genetics, and their antigenic characters are important aspects of myxoviruses which we are going to discuss and which are all going to have a bearing on my particular problem about classification, just as much as the purely anatomical aspect of the subject.

I hope that at the end of this meeting we are going to come away with beautiful clear notions of how myxoviruses are constructed, how they multiply, and what the relation is between their physiology and their rôle in nature.

REFERENCES

ANDREWES, C. H., BANG, F. B., and BURNET, F. M. (1955). *Virology*, **1**, 176.
BECKER, W. B. (1963). *Virology*, **26**, 318.
LWOFF, A., HORNE, R. W., and TOURNIER, P. (1962). *Cold Spr. Harb. Symp. quant. Biol.*, **27**, 51.
POLSON, A., and DEEKS, D. (1963). *J. Hyg. Camb.*, **61**, 149.

THE STRUCTURE OF MYXOVIRUSES AND ITS BIOLOGICAL SIGNIFICANCE

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MYXOVIRUSES were defined by Andrewes, Bang and Burnet (1955) as viruses 60 to 200 mμ in diameter, which adsorb to red cells, elute from them enzymically, are sensitive to ether and give rise generally to respiratory infections. The members at that time were influenza A, B and C, fowl plague, Newcastle disease and mumps.

The early electron microscope studies were performed on unstained, positively stained or metal-shadowed preparations. Such work revealed that any of the then known myxoviruses could be spherical, filamentous or pleomorphic in form (Taylor *et al.*, 1943; Bang, 1946; Schäfer, Munk and Armbruster, 1952; Valentine and Isaacs, 1957). Bang (1955) has reviewed the earlier literature.

The viruses of Newcastle disease and mumps were found to be larger than those of influenza and fowl plague. Ether treatment of influenza, fowl plague, Newcastle disease and mumps viruses revealed distinct morphological structures which could be correlated with the H (or V) antigens and the s-antigens. The components associated with the H antigens consisted of material derived from the envelopes of the viruses and caused haemagglutination. The s-antigens—which were normally within the virus envelopes—contained the RNA and were demonstrable by complement fixation (Davenport, Rott and Schäfer, 1960; Schäfer and Zillig, 1954; Schäfer and Rott, 1959).

The introduction of the negative staining technique (Brenner and Horne, 1959) enabled the finer details of structure to be resolved, and it was immediately apparent that though all myxoviruses had the same basic construction of an envelope studded with projections enclosing a helically arranged nucleoprotein, there were two quite distinct morphological varieties. Each member of the group could be assigned on the grounds of appearance to one or other subgroup. The criteria for distinction are differences in (1) the overall size and shape of the particles, (2) the appearance of the surface projections, and (3) the dimensions of the ribonucleoprotein.

THE INFLUENZA GROUP

All viruses in this group have a structure similar to that of influenza virus (Fig. 1). The gross structure is of the compound helical type and consists of a loose envelope enclosing the ribonucleoprotein which is itself arranged helically. The particles vary from 800 Å to 1,100 Å in diameter, and though not of constant shape generally assume either roughly spherical or filamentous forms. The envelope, which is not rigid, consists of a distinct membrane from which arise cylindrical projections 90 Å long and 15–20 Å wide. These seem to be spaced fairly regularly though no particular pattern has yet been discerned. The particles are stable and do not disrupt spontaneously, so that in the intact particle no features of the internal component are seen. Classical descriptions of influenza virus were made by Horne and co-workers (1960).

Viruses within the group are not morphologically distinguishable from each other. The degree of pleomorphism varies occasionally between strains of the same virus but is generally insufficiently characteristic to be helpful, except in the case of certain filamentous strains (Choppin, 1963).

Before the components of the virus are discussed, some of the factors determining the morphology of the whole particle will be considered.

The components of these viruses are assembled at or near the cell surface immediately before they are released. The virion is formed only when its constituents have traversed the cell membrane (Morgan, Rose and Moore, 1956). In the course of this process some host-cell material is incorporated into the virus coat. This phenomenon has been studied immunologically by Smith,

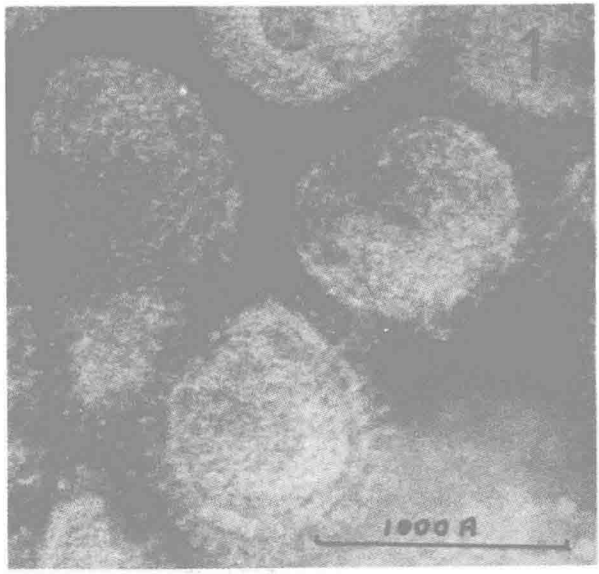

FIG. 1. Influenza A virus.

Belyavin and Sheffield (1953), cytologically by Hoyle (1954), chemically by Kates and co-workers (1961), and in the radioactive incorporation studies of Wecker (1957).

The study of filamentous influenza strains has given some insight into the mechanisms controlling gross virus structure. Influenza virus filaments were considered not to be infective until Valentine and Isaacs (1957) demonstrated ribonucleoprotein in such particles, though its arrangement in filaments was different from that in spheres. Choppin, Murphy and Tamm (1960) isolated a strain of

influenza A2 composed predominantly of filamentous virus which was as infective as other spherical strains isolated at the same time. Both forms contain RNA (Ada and Perry, 1958). The filamentous strains do, however, change rapidly to spherical forms on passage in eggs, without any change in the surface properties of the virus or in the infectivity of the preparation. Burnet and Lind (1957) were able to maintain a filamentous strain largely in this form by passaging at limit dilutions. They concluded that the change to spherical forms was mutational and perhaps similar in some respects to the *O-D* variation. Kilbourne (1963), summarizing the evidence, regards the morphology of influenza virus as a genetic trait which must, therefore, be coded by viral nucleic acid. Under certain well-defined conditions the morphology of the virus can be used as a marker in recombination studies.

Though morphology is determined predominantly by heredity other factors may influence this characteristic. Blough (1963*a*), using surface-active agents on host cells, produced apparently fully infectious filaments from spherical virus. It is possible that the inability of such cells as HeLa cells in culture to produce infectious virus, even though the virus components are formed within the cells, is due to the absence of budding at the cell membrane which normally occurs in allantoic cells (Hoyle, 1954).

Kilbourne and Murphy (1960) point out that there may be a relationship between filamentous virus forms and biological characteristics in that these strains have low rates of multiplication and low yields compared to spherical forms.

The most positive correlation between the whole virus structure and infectivity occurs with incomplete virus. This phenomenon has been studied in the electron microscope in shadowed preparations (Paucker, Birch-Andersen and von Magnus, 1958) and by negative contrast methods (Moore *et al.*, 1962; Barry, Horne and Waterson, 1962; Morgan, Hsu and Rose, 1962). Incomplete virus particles are irregular in outline and pleomorphic, and the particles appear less dense than those of the normal virus. Surface

structures have the same appearance as on normal particles; incomplete virus retains normal surface biological properties. Serologically the s-antigen is in greatly reduced amounts (Rott and Schäfer, 1961*a*) and the internal component from incomplete virus has not been seen by negative staining methods. Paucker and co-workers (1958), using the metal-shadowing technique, stated that it was similar in appearance to the normal but in much reduced quantity. The reasons for the altered morphology are ill-understood. The von Magnus phenomenon is probably a genetic phenomenon, though evidence for this is based upon its relationship to multiplicity events, the known ability of influenza virus to undergo genetic events, and the failure of myxoviruses in which recombination cannot be demonstrated to undergo the von Magnus phenomenon (Barry, 1961).

Components obtained after ether treatment of the virus

The influenza virus envelope contains both lipid and protein (Hoyle, 1952). Treatment with lipid solvents such as ether or with detergents (Laver, 1963) causes the disintegration of the coat with the release of the ribonucleoprotein and total loss of infectivity. A variety of methods is available for the separation of the surface components from the internal helix.

The envelope material retains the properties of the virus surface. These are the haemagglutinating activity, the enzyme neuraminidase and the antigen responsible for the production of the haemagglutination inhibiting and the neutralizing antibody. Morphologically the coat material breaks up into small rosette-like structures about 350 Å in diameter consisting of the surface projections attached in a radial fashion to small pieces of surface membrane (Fig. 2).

Noll, Aoyagi and Orlando (1961) found that 5–15 per cent of the virus protein was neuraminidase and that removal of 95 per cent of the enzyme did not produce any visible change in the surface structures. Using γ-chymotrypsin to release the enzyme