Thirty years of biochemical work on foot-and-mouth disease virus at the Pirbright Institute

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Summary: Creation of the Biochemistry Department at the Pirbright Institute in 1954 coincided with the expansion of molecular biology. Research concentrated on the detailed structure of the aphthovirus genome and capsid proteins, particularly VP1. Another line of research was the amino acid sequence which elicits the formation of neutralizing antibody. New knowledge of the structure of the virus is important for the development of new vaccines, particularly those derived by genetic intervention.


The celebration of an Institute’s work over a period of six decades is an occasion for nostalgia and reflection. In the previous paper John Brooksby described the Institute’s role in foot-and-mouth disease research from its beginning in the 1920’s. Since my association with the Institute began as recently as 1955 my first-hand knowledge of its work is much shorter. It is also much narrower because in his position as Director for the greater part of two decades and also because of his direct confrontation with the disease in the field, where it really counts, John Brooksby saw a much wider scene than I did. Consequently I shall confine my presentation to work with which I am most familiar, mainly that of the Biochemistry Department during the last 30 years. This occasion also gives me the opportunity to refer to the work of the individuals within the Department during that period and to thank them and many colleagues in other Departments for their cooperation and for teaching me about animal disease.

During the last 30 years virology has changed considerably. It has ceased to be the preserve of the medical and veterinary professions and has become an area of considerable interest and importance to many other branches of science, stimulated by the explosive expansion of work on the molecular aspects of biology during this period. I was fortunate enough to join the Institute’s staff when the impact of molecular biology on virology was just starting to be felt. I was doubly fortunate in that the Director of the Institute at that time, Dr Ian Galloway, had already realised the importance of the fundamental sciences in the study of viruses. Ian Galloway gave me a gentle prod in the direction of nucleic acids, this at a time when not

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everyone was willing to accept their central role in all areas of biology, including virology.

**EARLY FRACTIONATION STUDIES**

The overriding theme of the Department’s work since its creation in 1954 has been to relate the structure of viruses to their function and to attempt to explain their properties in chemical terms. Since the role of the Institute was to study the exotic virus diseases of farm animals, particularly foot-and-mouth disease, it was clear that we should try to bring some chemical understanding to the problems associated with those diseases. One of these was to define what was required of a vaccine against foot-and-mouth disease. At that time, vaccination against the disease on a large scale had become feasible by the development, principally by Frenkel in Holland, of large-scale production of the virus in fragments of tongue epithelium. Fractionation of virus harvests by centrifugation had revealed the presence of a sub-viral particle in addition to the infectious virus particle. Joan Crick and I showed that most of the immunogenic activity was associated with the virus particle. We also showed that treatment of a virus harvest so that it was no longer infectious, always a tricky and dubious procedure when the time-honoured formaldehyde was used, could be achieved with greater certainty and consistency by using acetyleneimine. Imines are now used by most manufacturers of foot-and-mouth disease vaccines.

The early fractionation studies were at best rudimentary and it was clearly desirable that we should need to learn about the structure of the virus if we were to understand its biology. The work of Bradish and Brooksby at Pirbright and workers in other foot-and-mouth disease institutes had shown that the virus was small (c 25 nm) and spherical. However, it was not known at that time whether it contained DNA or RNA and it was the work with Doreen Stewart at the Institute and Mussgay and Strohmaier in Tübingen in 1958 which showed that the virus contained RNA. The RNA which could be obtained either by extraction with phenol or simply by reducing the pH below 7 was infectious and this property enabled us to do many experiments on the attachment of the virus to susceptible cells and on its replication before we had any firm knowledge of its protein composition.

**KNOWLEDGE OF IMMUNOGENIC SITES**

Purification of the virus was clearly an important target and this was achieved in 1963 in experiments with Cartwright. Once we had purified particles at our disposal, it was possible to make a thorough analysis of the virus. It soon became apparent that foot-and-mouth disease virus had many properties in common with poliovirus, the common cold virus and encephalomyocarditis virus and indeed each of these viruses is a representative member of the four genera of the family Picornaviridae. All the viruses contain four proteins of similar molecular weight (three with Mw of c 25000 and one with Mw of c 10000). Foot-and-mouth disease virus possessed the interesting property of losing its ability to attach to susceptible cells and hence its infectivity when treated with trypsin. Burroughs showed that this was caused by cleavage of only VP1 of the four virus proteins, the others apparently being unaffected. More significantly, this treatment also severely impaired the abil-
lity of viruses of serotype O to elicit neutralizing antibody and protect animals against infection. This work was complemented with the observations which Smale and I made that the trypsin-treated virus would not react with early (IgM) antibody, although it still complexed with late (IgG) antibody. This effect was demonstrated quite strikingly in the electron microscope and our tentative conclusion was that the sites on the virus to which the IgM molecules attached appeared to be at the apices of the icosahedral particle.

The observation by Burroughs was critical to the development of our understanding of the immunogenicity of the virus and led directly to the concept of a genetically engineered vaccine. Thus Laporte and his colleagues in France showed that VP1 alone would elicit the formation of neutralizing antibody and it has since been shown by several groups that animals can be protected by injection of this protein alone. By carrying the dissection of the virus a stage further, Rowlands and I showed in collaborative experiments with Lerner’s group at the Scripps Clinic in California that a synthetic peptide comprising 20 amino acids and containing the site on VP1 which is cleaved by trypsin will elicit the formation of neutralizing antibody and will protect cattle against challenge infection. Clearly at this stage it is difficult to say whether peptides will become the next generation of foot-and-mouth vaccines. Whether they do or not, there can be no doubting the value of the peptide approach in reaching an understanding of the immunogenic sites of the virus, particularly with regard to antigenic variation.

**IDENTIFICATION OF DIFFERENT SEROTYPES**

One of the most difficult problems in protecting animals against foot-and-mouth disease by vaccination is the occurrence of the virus as seven serotypes with many antigenic variants among the serotypes. In practical terms this means that a wide range of vaccines must be available, a problem which presents considerable difficulties to control authorities and manufacturers alike. The application of sequencing techniques for nucleic acids has now made available the derived amino acid sequences of the virus proteins of isolates belonging to different serotypes and subtypes and from this information the chemical basis of antigenic variation is being worked out at the most fundamental level. There seem to be real hopes that this information will lead to hybrid peptides which would afford wider antigenic protection.

An important bonus from the work on the RNA of the virus has been its value in the identification of viruses causing outbreaks in the field. The time-honoured methods for diagnosis rely on the reaction between the virus and its specific antibody and there is little doubt that these will continue to be used. However, there are instances when it is necessary to distinguish between isolates which are closely related serologically. Such an occasion arose in 1981 when Underwood proved decisively by oligonucleotide fingerprinting of the virus RNA that the outbreak on the Isle of Wight (incidentally the most recent outbreak in the United Kingdom) was caused by a virus which was indistinguishable from that causing outbreaks in Jersey and in Normandy and Brittany and from the virus used for preparing the vaccines in use in those areas of France at that time. Since 1981 the same method of identification has been used in similar outbreaks in other parts of the world.

Studies on the replication of the virus have been made over a long period. Replication of the RNA clearly involves a double-stranded replicative intermediate and
certain of its features led Martin and me to propose a cyclic model for the intermediate. This model still holds attractions and provides an explanation for some of the properties of the replicative intermediate and replicative form which cannot be deduced from the more conventional model.

MAPPING EXPERIMENTS ON THE VIRAL GENOME

Clearly if we were to progress with relating structure to function it was next necessary to construct a biochemical map of the genome and the work of Sangar, Black and Rowlands in the mid 1970's provided such information. The map was similar to those of other picornaviruses such as polio and rhino virus but differed in one important respect. Four primary products are obtained in FMDV-infected cells compared with the three obtained with the other viruses. Moreover, there was an interesting structural difference in the RNAs of FMDV and encephalomyocarditis virus (EMCV) on the one hand and those of polio and rhino virus on the other. In collaboration with Fellner at the Searle Laboratories, Newman and Harris showed that FMDV, like EMCV, contains a polycytidylic acid tract near the 5' end of the genome. The length of this tract varies between different isolates of both viruses but it is located at a specific position, 150 bases from the 5'-terminus of EMCV RNA and 390 bases from the 5'-terminus of FMDV RNA. The role of this tract has not been defined. The limit of our knowledge is that removal of the short length of bases to the 5' side of the polycytidylic acid tract destroys the infectivity of the RNA without impairing its ability to produce in a cell-free system the full complement of virus-induced proteins obtained with the full length RNA.

The mapping experiments pinpointed the positions of the capsid proteins and, in conjunction with the proteolytic cleavage experiments, were crucial in the subsequent recombinant DNA and nucleic acid sequencing experiments. In addition, however, they provided important information regarding the location of the polymerase and protease genes and the region coding for the small protein which is linked to the 5' end of the RNA.

In addition to their purely practical value, in terms of potential peptide vaccines, the sequencing work has provided us with an insight into the evolution of the virus. The experiments of Harris and Robson using the RNA:RNA hybridisation technique showed that the seven serotypes of the virus could be divided into two groups, one comprising the serotypes A, O, C and Asia 1, and the other consisting of three serotypes from the Southern African Territories. As sequencing techniques were applied to the study of the viruses it became clear that the subdivision into two groups was valid, even to the detail that only the Southern African serotypes possess a cysteine at the C terminus of VP1. Moreover the availability of sequences for the capsid proteins has provided us with a chemical explanation for the lability of the immunogenicity of viruses of some serotypes to proteolytic enzyme cleavage, whereas others are insensitive. The amount of basic chemical information which can be gathered in a relatively short time now means that answers to questions which we have posed for 30 years are now within our grasp.

FUTURE PROSPECTS

At the beginning of this talk, I spoke about the nostalgia associated with an occasion such as this. I also pointed out that on such occasions it is appropriate to
reflect on the work that has gone on during that period. I hope that what I have said does justice to the efforts of all those who have worked in the Biochemistry Department since its formation in the mid 1950’s. However, an occasion such as this is also a time to look forward. In the difficult times for science in general, agricultural science appears to have been selected by Government for the unkindest cuts of all. Clearly such a policy has a major impact on the Pirbright Institute. It is just possible that the great success of the agricultural industry in Europe, and in the United Kingdom in particular, is unduly influencing Governmental policy. However, the Institute has a fine record of cooperation with the underdeveloped countries and the diseases which have been the Institute’s concern throughout its history are still rife in those countries. The Institute still has a major role to play in the control of virus diseases of farm animals, whether by epidemiological studies or by sophisticated science in the test tube. Both branches of science have a part to play and I am proud that I have been part of it for so long.

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TRENTE ANS DE RECHERCHES BIOCHIMIQUES SUR LE VIRUS APHTEUX A L’INSTITUT DE PIRBRIGHT. — F. Brown.

Résumé : La création du Département de biochimie à l’Institut de Pirbright en 1954 a coïncidé avec l’expansion de la biologie moléculaire. Les recherches ont consisté essentiellement à décrire de façon de plus en plus détaillée la structure du virus aphteux : le génome à base d’ARN et les protéines de la capside, parmi lesquelles la protéine VP1 joue un rôle déterminant. Les chercheurs ont mis en évidence la séquence d’acides aminés qui assure la capacité de réplication de l’ARN et celle qui comporte les sites immunogènes. Les connaissances acquises sur la structure du virus aphteux sont très importantes pour la conception des vaccins, notamment par génie génétique.


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TREINTA AÑOS DE INVESTIGACIONES BIOQUÍMICAS DEL VIRUS AFTOSO EN EL INSTITUTO DE PIRBRIGHT. — F. Brown.

Resumen : La creación del Departamento de bioquímica en el Instituto de Pirbright en 1954 coincidió con la expansión de la biología molecular. Las investigaciones consistieron fundamentalmente en describir de modo cada vez más detallado la estructura del virus aftoso: el genoma a base de ARN y las proteínas de la cápsida, entre las cuales la proteína VP1 desempeña un papel determinante. Los investigadores evidenciaron la secuencia de ácidos aminados que asegura la capacidad de replicación del ARN y la que incluye los lugares inmu-
nógenos. Los conocimientos adquiridos sobre la estructura del virus aftoso son muy importantes para la concepción de las vacunas, especialmente por ingeniería genética.