Roy Markham

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ROY MARKHAM: PIONEER IN PLANT PATHOLOGY

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INTRODUCTION

I first met Roy Markham in September 1945, when I came to Cambridge after four years in the New Zealand army to begin research for the PhD in plant virology. As far as the University was concerned Dr. K. M. Smith was my supervisor, but I very rapidly came under Roy's influence, and he was my de facto supervisor for three years. I came back to his group for four full years from 1952 to 1956 and later made several short visits to his laboratory both in Cambridge and, after the shift, in Norwich. Thus, I knew him well for a significant portion of his working life.

Roy made a number of seminal contributions to plant virology and thus to plant pathology. However, he was in no sense a plant pathologist himself. His formal training was in biochemistry, and he learned a great deal in the physical sciences on his own account. In the main thrust of his research, he was really one of the pioneers in that branch of science now known as molecular biology.

EARLY LIFE AND EDUCATION

Roy Markham was born on 29 January, 1916, the son of a London stockbroker, A. C. C. Markham. He was sent to a series of schools, including, for a period, a day school in Magdeburg where he became proficient in the German language. He later put this ability to good use by reading the early literature in German on the organic chemistry of the purines, pyrimidines, and nucleic acids.

He appears to have been an unhappy student during the early years of his education, but going to St Paul's School in London in 1931 led to a marked

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change. He became interested in several subjects, especially physics and chemistry, and became an industrious student. During this period he decided to become a scientist and soon became interested in the practical aspects of science. He made himself a workshop-laboratory in his home where he experimented in chemistry, optics, photography, and the construction of radios. The desire to work with his own hands never left him, and in all his working life he never had a personal technical assistant. Even when he became director of the John Innes Institute, he was often to be found in the workshop.

Roy did not excel in any particular sport but participated actively in several. He was a forward in St Paul's fourth rugby team and in the second team of his college at Cambridge. He enjoyed swimming and squash and played tennis until about 1957.

He was admitted to Christ's College, Cambridge, in October, 1935. At first he wanted to be a bacteriologist. Because bacteriology was taught as part of pathology in the Medical School, he took courses in pathology, biochemistry, physiology, and zoology during his first two undergraduate years. He became less than enthusiastic about the courses in pathology and decided to study biochemistry for the second part of his degree. During this period, the biochemistry department was headed by Sir Frederick Hopkins and was staffed by a diverse group of talented individuals that included N. W. Pirie. The latter taught the first four weeks of the practical classes. This meeting with Pirie was important for Roy's development in both philosophical and practical ways. Roy appreciated Pirie's highly critical approach both to research and to scientists themselves, and indeed he followed in Pirie's footsteps in this respect.

Two years earlier, Pirie, together with F. C. Bawden, had shown that tobacco mosaic virus was a ribonucleoprotein, rather than a protein as Stanley had claimed. They had also isolated tomato bushy stunt virus in crystalline form and had shown that it was also a ribonucleoprotein. These were exciting findings for all of biology, and while Pirie's student, Roy decided to become a plant virologist. In 1938, he obtained an upper second class pass in his undergraduate degree, and Pirie took him on as a research student.

PhD RESEARCH

During his time with Pirie, Roy gave many the impression that he had a dilettante approach to research. He spent much time in the workshops of the Biochemistry and Anatomy Departments attempting to make small high speed airdriven centrifuge rotors to be used in isolating viruses. These efforts were without practical success. However, in another undertaking he was highly successful. He designed an ingenious and versatile steam microdistillation

apparatus. This became known as the Markham still and was widely used for many years as standard equipment for the estimation of nitrogen by the micro-Kjeldahl method.

Roy worked under Pirie's loose form of supervision until the middle of 1940. During this period he was supported financially by his family. However, in the early summer of 1940 a post became available as assistant to K. M. Smith, who was director of the Plant Virus Station, at that time connected with the School of Agriculture, Cambridge. On Pirie's advice, Smith offered Roy the job, which he accepted. He began work in July 1940. In September of that year, he married Margaret Mullen whom he had known since they were both in their early teens.

K. M. Smith had arranged with D. Keilin, the director of the Molteno Institute for Biology and Parasitology, for the staff of the Plant Virus Station to occupy a small laboratory space on the top floor of the Institute. In addition, Smith and his staff had the use of glasshouses and sheds situated on the University farm on the outskirts of the town.

By the end of 1940, Roy had seriously begun research for his PhD which was awarded to him in 1944. During this period he had two tasks which he kept quite separate: his work as Smith's assistant, and his doctoral research. He learned the then-current practical plant virological methods from Smith and carried out diagnostic work and routine monitoring of virus-free stocks of potatoes. He was also responsible for photography for the station. For his PhD research, he devised a program for studying the physical chemistry of large proteins, mainly of plant viruses. He carried out this research with virtually no relevant formal undergraduate training and without supervision, since Pirie had moved to Rothamsted and K. M. Smith was strictly a biologist. His PhD thesis describes three essentially separate investigations. First he determined the diffusion constant of tomato bushy stunt virus and the viscosity of solutions of the virus using published procedures. From these data he calculated the molecular mass of the virus to be $10.5–10.8 \times 10^6$, quite close to the current value of 8.9×10^6.

In the 1940s, high speed angle or swinging bucket rotors suitable for the preparative sedimentation of viruses were not available, but the Sharples centrifuge was installed in some laboratories. This centrifuge consisted of a narrow, hollow cylindrical rotor through which the solution of particles could pass in a continuous flow. In a technique devised by Schlesinger, sedimenting molecules were trapped in a layer of agar lining the cylinder. In his second project, Roy simplified use of this device by replacing the agar with a layer of filter paper, from which the virus could be more easily extracted. Using this procedure, he sedimented tobacco mosaic virus, tomato bushy stunt virus, potato virus X, tobacco ringspot virus, tobacco necrosis virus, and two bacterial viruses. He further showed how the Sharples centrifuge could be

used to measure the molecular mass of large molecules. One measured the concentration remaining in solution after a sedimentation period under defined conditions.

In his third project, he used the Sharples rotor to isolate tobacco mosiac virus without salt precipitation. He then studied the effect of salting-out on the degree of aggregation and the infectivity of the preparations. End-to-end aggregation was measured by viscosity. He showed clearly that as end-to-end aggregation increased, infectivity was reduced, and that infectivity could be restored by disaggregation.

TURNIP YELLOW MOSAIC VIRUS

The first field sample of a turnip plant infected with turnip yellow mosiac virus (TYMV) was sent to K. M. Smith in the early 1940s, but serious work on this virus did not begin until early in 1945. Smith studied biological aspects of the virus, the disease, and the question of insect transmission. Roy worked out a method for isolating the virus and carried out biophysical and biochemical investigations. This collaboration, which lasted about three years, was their most fruitful. Soon after, Smith turned more to the study of insect viruses, while Markham began his work on the biochemistry of RNAs.

When I arrived at the Molteno Institute towards the end of 1945, Roy had already succeeded in obtaining purified preparations of TYMV in crystalline form as octahedra. Not long afterwards, through use of the analytical ultracentrifuge at Oxford, he found that these purified preparations contained two sedimenting components. He further found that when concentrated solutions of the virus were subjected to centrifugation in a celluloid tube in an angle rotor, and the process halted before sedimentation was complete, two boundaries could be observed. By repeated cycles of incomplete sedimentation, he was able to isolate the two components in almost pure form. He called them top (T) and bottom (B) components. He went on to show that the B component contained about 34% of RNA and was infectious, whereas the T component contained no RNA and was not infectious. The electrophoretic mobilities of the two types of particle were identical. During my own PhD research I had developed an interest in the application of serological techniques to plant viruses. I was able to show that the T and B components appeared to be identical by any available serological test.

Consideration of these properties of the T and B components led to two conclusions that were important for all virology. First, the fact that the nucleoprotein particles were infectious, whereas the protein particles were not, was the first demonstration for any virus that the viral nucleic acid is essential for infectivity (12). It did not, of course, eliminate the possibility that the protein also had an essential role. However, it undoubtedly focused

Roy's interest on viral RNAs, for these were the next subject to which he directed his attention.

The second important conclusion, which concerned virus structure, was arrived at by Markham in 1951 (11). He deduced that the RNA of TYMV is located within a shell of protein. The observations that led him to this view were as follows: (*a*) The surface of the particle with 34% of RNA appeared to be identical to that of the particle with no RNA, because the electrophoretic mobilities of the two classes of particle were the same, because they behaved identically with respect to the binding of rabbit antibodies, and because they formed mixed crystals. (*b*) The diameters of the two classes of particle were the same. If any significant amount of RNA lay outside the protein, then the nucleoprotein should have had a larger diameter. Recent low-angle neutron scattering studies (10) have shown that there is little penetration by the viral RNA into the surrounding protein shell, which confirms closely Markham's picture of the virus structure.

This understanding of the distribution of protein and RNA in the TYMV particle and the observation that the RNA was necessary for infectivity were important experimental findings that led Crick & Watson (6) to propose that the protein of all small isometric viruses forms a protective shell for the nucleic acid.

In 1948, before I left his laboratory, Roy had already reached the conclusions stated in his 1951 paper, and I shall digress for a moment to tell a personal anecdote. Elsden (7) has suggested that Roy developed a lasting love of plants and of gardening while working with K. M. Smith. I do not think that this was the real situation. He worked with plants only because he regarded them as very convenient hosts for growing viruses, compared to the animal systems of the period. He had little intrinsic interest in the plants themselves. His wife Margaret, on the other hand, had always had a deep love of plants and things horticultural and has always maintained a fine English garden. Roy supported her strongly in this enterprise, but did so for her sake and not for the sake of the plants. In fact, he rather despised botany as an academic subject. As part of my PhD work, I took advanced undergraduate courses in several departments of Cambridge University, including the Department of Botany. Roy was quite disparaging of this interest of mine and was particularly sarcastic about anything having to do with morphology. Thus, when he first propounded his ideas in the laboratory concerning the structure of TYMV, I suggested that he might go down in history as a famous virus morphologist. It was one of the few times that he was truly angry with me.

One missed opportunity in Roy's work with TYMV should be mentioned. This virus, with its two sedimenting components, would have been an ideal model system with which to develop the use of density gradients for the

separation of macromolecules. Roy probably had a better understanding of the physical processes involved in sedimentation than anyone else working with viruses during this period, but he never attempted to use gradients, probably because he thought the viscosity of the medium would be a big problem. It was left to Brakke (5) to introduce density gradient fractionation, a technique which has since had great importance in virology and molecular biology.

RIBONUCLEIC ACIDS

In 1948, J. D. Smith joined the staff of what had come to be called the Agricultural Research Council Virus Research Unit. He and Roy began a study of the structure and biochemistry of ribonucleic acids. For much of their work they used RNA isolated from TYMV. Over the next four years, they made many technical advances for the separation and detection of ribonucleic acid components by means of paper chromatography and paper electrophoresis and made significant contributions to our understanding of the structure and composition of ribonucleic acids generally. They elucidated the mode of action of pancreatic ribonuclease and provided key information leading to an understanding of the chemical structure of the phosphodiester backbone of RNA. About 1952, J. D. Smith moved into investigation of bacterial viruses, but for some years thereafter, many visitors came to the laboratory for shorter or longer periods to learn techniques for studying RNA and how to apply them to their particular interests.

In spite of all the contributions concerning viral RNAs that were made in Roy's laboratory, there was one lost opportunity that in retrospect is difficult to understand. In a laboratory where preparations of viral RNA were being made almost daily from 1948 to 1956, how was it that no one attempted to test the infectivity of the product? As far as I am aware, such an experiment was not even mooted. In spite of the infectivity results with the two classes of TYMV particle, the idea persisted that the protein might also have an essential function for infectivity. It was left to Gierer and Schramm in 1956 (8) to demonstrate the infectivity of tobacco mosaic virus RNA.

Although there was a succession of visitors to his laboratory in the 1950s, Roy never built up a team of PhD students and postdoctoral researchers, the sort of group that would be expected to form around an American scientist of his abilities. In fact, he rather despised the United States until he visited that country for the first time about May 1955. This leads me to the question of accents.

Roy was fond of teasing others in the laboratory about the way they spoke. In my case, he joked about my New Zealand accent. On many occasions I suggested that he also had an accent, that of an English public school. He claimed his speech was accentless, until he went to the United States. While

there, he made a tape recording of his news and sent it home to Margaret. When he returned, we played the tape for him. He was quite astonished at the sound of his own voice and never again claimed that he spoke without accent.

Another incident that I recall from this period illustrates both Roy's taste in music and his quick-wittedness in one-upping other people when he felt like doing so. A visiting Italian plant virologist working in the laboratory asked Roy if he liked Italian opera, to which Roy replied, "Yes, very much." "Which is your favorite composer?" was the next question. Roy replied: "Mozart."

An anecdote recorded by James Watson in *The Double Helix* (16) illustrates not only Roy's frequent desire to be one-up in conversation, but also his willingness to help others engaged seriously in research. Watson was in search of some free TMV for X-ray crystallographic studies: "I thus went to Roy Markham to see if any spare TMV was on hand. Markham then worked in the Molteno Institute, which unlike all other Cambridge labs, was well heated. This unusual state came from the asthma of David Keilin, then the Quick Professor and Director of the Molteno. I always welcomed an excuse to exist momentarily at 70°F even though I was never sure when Markham would start the conversation by saying how bad I looked, implying that if I had been brought up on English beer I would not be in my sorry state. This time he was unexpectedly sympathetic and without hesitation volunteered some virus. The idea of Francis and me dirtying our hands with experiments brought unconcealed amusement."

OTHER PLANT VIRUSES

Towards the end of the 1950s Roy lost interest in RNAs as such, although there was still a very great deal to be learned about them. He returned to his former interest in virus structure and composition and, often in collaboration with a visitor to his laboratory, carried out experiments on a number of different plant viruses. For example, in collaboration with R. H. Symons from Australia and two members of the laboratory staff, he compared six field isolates of TYMV with respect to the base composition of the RNA, the frequency of certain oligonucleotides found following digestion of the RNAs with pancreatic RNAase, and the amino acid composition of the coat protein (15). By all three criteria, the six strains fell clearly into two groups. This was the first occasion on which detailed chemical data from both protein and RNA studies were used to compare a set of naturally occurring virus strains. L. M. Black brought his wound tumor virus to Cambridge, and together he and Roy carried out some of the earliest studies of the properties of the double-stranded RNA of this virus (1, 2). They also studied the ultrastructure of potato yellow dwarf virus (3).

With other colleagues, Roy studied the structure or structural components of cowpea chlorotic mottle virus (4) and alfalfa mosaic virus (9). Working with R. Hull and I. R. Schneider, he used the analytical ultracentrifuge to show that the satellite RNA of tobacco ringspot virus was encapsidated in shells of the virus protein. Roy and his coresearchers found that there were about 12–25 satellite molecules in different shells, which yielded a whole series of components when virus preparations were banded in cesium chloride gradients (14).

During the 1960s and 1970s, one of Roy's major interests was in improving methods for obtaining information on virus architecture using the electron microscope. In particular, he recognized that there was structural information in negatively stained electron micrographs of virus particles that could not be grasped by the naked eye because of the background noise. He therefore set about developing a relatively simple homemade apparatus for extracting information about repeating structures in electron micrographs of both isometric and rod-shaped structures (13).

At the same time, another method for extracting detail from electron micrographs based on optical diffraction was proposed by H. S. Lipson and C. A. Taylor (7). A. Klug was stimulated by the improved detail obtained by Markham's "averaging" methods. He developed the optical diffraction technique using a low power laser light source. During the 1960s, much more powerful methods of image processing were developed, but Roy did not take part in these advances. In fact, he did not have a specially designed laboratory for this type of work until he moved to the John Innes Institute in 1967. He designed this laboratory and had the master builder align the bench supports using a laser beam. This was one of the first uses of lasers in building.

ADMINISTRATION

In 1960, Roy succeeded Kenneth Smith as Director of the A.R.C. Virus Research Unit. Some laboratory space had already been built at the site of the glasshouses on the Cambridge University Farm. Under Roy's guidance, additional quite large laboratories and new glasshouses were built on the site, and Roy took great pride in having provided these improved facilities for the workers in the Unit. In this period, he did a great deal to foster research on plant viruses by others.

In the early 1960s, the upper level administrators of agricultural research in England developed concerns that were to affect Roy's future. With a fine tradition in horticultural research going back to 1911, the John Innes Institute was considered to be too isolated at its country site at Bayfordbury. The trustees of the Institute considered a move to a site near the University of East Anglia in Norwich. The move was finally approved by the trustees, the

University of East Anglia, and the A.R.C. However, in 1966 the director of the Institute, K. S. Dodds, resigned, and a replacement was needed to supervise the move of the Institute and its establishment in a new environment. Roy was not by nature a keen administrator, but he was, I think, pressured by the A.R.C. to accept the directorship. He did so on the condition that he could bring the staff of the Virus Research Unit with him. This condition was accepted, and he was appointed director in June 1967. He also held a post in the University of East Anglia as John Innes Professor of Cell Biology. In his first years in this new post, Markham faced many important decisions and difficult tasks: the siting of the new Institute; planning new laboratories and glasshouses; and, not least, bringing together two somewhat disparate staff groups—the old John Innes group from Bayfordbury, and the staff that came from the Virus Unit in Cambridge—and creating an ethos for them.

Roy's attitude to the recruitment of new staff, and indeed to the administration of research generally, is well illustrated by a final paragraph he wrote in a letter to Dr. R. Hull at Wye College, following the latter's appointment to the A.R.C. Virus Research Unit in 1965: "You will note from your Contract of Employment that your hours of work are nominally 43 per week. My main objective in running a research unit is to provide workers with as much in the way of facilities for indulging their interests as I can, and my hope is that people will be interested enough to use them to their full extent. I am also of the opinion that half an hour's good thinking in the bath qualifies as an hour of work. Yours sincerely, Roy Markham."

Roy took a particular interest in ensuring that there were amenities for the staff at the new Institute, including a club building with a cafeteria and a swimming pool. Roy was sitting in the cafeteria one day with an earnest American professor who saw the room filling up and asked, "Say, how many people work here?" Roy replied, "One or two, but we employ 160." In the Institute there is also a fine lecture hall and a small library of rare and valuable old books on horticulture and the plant sciences generally. This library has grown out of the original collection of William Bateson, the first director of the John Innes Institute.

On its new site, the Institute was organized in four departments: Cell Biology, Genetics, Applied Genetics, and Ultrastructural Studies. Under Roy's oversight, the Institute grew and rapidly established itself as a center producing first-class research in many aspects of plant science. From the start, plant virology was a strong component of the research that was built around the group brought from the Unit in Cambridge. Over the years, members have been lost from the original staff of the virus unit by death or retirement, but excellent younger scientists have been appointed to succeed them. Roy himself died after a long illness on 16 November, 1979. Were he alive today,

he would be very happy with the quality of the research in fundamental plant virology that continues to issue from the Institute he did so much to reestablish in its new environment.

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