Interferon: the early days

A fortunate job offer as a young man led **Derek C. Burke** to work at the NIMR during the exciting period in the late 1950s when interferon was just being discovered.

Fifty years ago I was lucky enough to be a young scientist working with Alick Isaacs, the discoverer of interferon. I first met Alick in November 1955, and he immediately impressed me as an extremely intelligent and very lively person. Little did I know that those next few years working with him were going to set the course of my whole career. I had just come back from the US, after spending 2 years as a postdoc at Yale, where I'd been working with some novel nucleosides from a Caribbean sponge, containing arabinose not ribose. I had a first degree in chemistry and a PhD for work on steroids, but the time at Yale was the start of my life-long fascination with the biological sciences. I had gone to the US in September 1953, and on the boat was a young man named Jim Watson, who had just published with Francis Crick that famous letter in *Nature*. I returned in 1955 on the *Mauretania*, newly married to a Yale PhD, liable for military service in the British army and without a job. So I was grateful to be offered two jobs in Britain – one working on rocket fuel development, and the other on the biochemistry of viruses at the National Institute for Medical Research in North London, which I gladly accepted. This was a 3-year appointment in the Chemistry Division, *not* the Virology Division, since Sir Christopher Andrewes, Head of Virology, allowed only medics into his division!

Interferon is discovered

My first project was to determine the nucleic acid content of influenza virus, known to be an RNA virus, but how much RNA? Near its end, Alick suggested that I should help him 'with something interesting that we are doing on interference'. 'We' was Jean Lindenmann and himself, it was March 1957, and interferon was only a few weeks old. The name was new – Alick once explained that it was 'time that biologists had a fundamental particle, for the physicists have so many: electron, neutron, proton, etc.' That did not stop Lord Hailsham, then Chairman of the MRC, objecting to such a nasty hybrid word – with both Latin and Greek roots! By then, though, the name had stuck.

Interferon had been discovered when testing quite another hypothesis. It was the steam age of virology (as Sir Christopher would say, referring rather disparagingly to the dream age that would follow – molecular biology and all that!), and no one really knew how animal viruses worked – indeed it was suggested that the viral coat was left outside the cell, like phage. Alick and Jean tested this by seeing whether any viral property – and they chose interference – was still associated with the outer membrane of the cells of the chick chorio-allantoic membrane, and could be washed off. What they found, of course, was not the viral coat outside the cell, but the interferon newly made inside the cell.

The system was crude. The virus used to stimulate interferon, heat-inactivated influenza virus, was not very potent. Interferon was estimated by challenging treated chick cells with infectious influenza virus and then measuring virus growth by haemagglutination titration. We tested, in sextuplicate, at least three twofold dilutions of the interferon sample; the amount of virus produced was measured by diluting it in serial twofold steps in plastic plates, and adding chicken red blood cells. The endpoint of the titration was the well with partial agglutination, and the reciprocal of the interferon dilution, the interferon titre. The experiments took hours to titrate,

◀ The entrance to the NIMR, Mill Hill, London, where the author worked with Alick Isaacs on interferon in the late 1950s. *James King-Holmes / Science Photo Library*

▶ A young Derek Burke cutting up fertile hens' eggs for interferon assays, with the assistance of Valerie Carver. *Derek Burke*

◀ Alick Isaacs (left) photographed in 1957, the year in which he and his Swiss colleague Jean Lindenmann (right, photographed in London in 1956) discovered interferon. Alick Isaacs was Head of the Laboratory for Research on Interferon at the National Institute for Medical Research from 1964 and was elected an FRS in 1966, shortly before his untimely death at the age of 45 in 1967. *Jean Lindenmann, Zürich, Switzerland*

involving little more than purely mechanical operations, and this left time to talk. Alick was the leader in conversation, and ideas for new experiments, political discussion or identification of snatches of opera that he would sing made the time pass quickly.

Characterizing the compound

Two papers had already been written for the *Proceedings of the Royal Society*, but there was still much to do. So we worked quickly and published the results in a series of papers in the *British Journal of Experimental Pathology*. I still have my laboratory notebooks, and my first experiment, dated 4 March 1957, was headed 'Dialysis of interferon' – we did not even know whether interferon would pass through a dialysis membrane! The second experiment, started the same day, was to test whether interferon activity was destroyed by shaking a crude preparation with ether. It was; another hint that interferon was a macromolecule. Next we tested the stability of interferon at different pHs and then a series of experiments to see if it behaved like a macromolecule, either a polysaccharide or a protein. It was precipitated with ammonium sulfate, degraded by treatment with the proteolytic enzymes trypsin and pepsin, inactivated by shaking with butanol, but not inactivated with periodate – all suggesting it was protein rather than polysaccharide, and if a protein, then presumably it could be purified, possibly relatively easily. The first of these conclusions was true, but the second took a long time and was much more difficult. The first paper in the series 'Studies on the production, mode of action and properties of interferon' was submitted as early as 23 July 1957. Alick wrote papers very quickly, taking the laboratory notebooks home and producing a first draft by the next morning.

The next paper, submitted on 7 November, described the use of ultraviolet-inactivated (UV) virus to induce interferon. We found that the time of irradiation was important, short periods producing high yields, while longer periods led to a complete loss of effectiveness. These observations are now most readily interpreted as a measure of the capacity of the virus to form double-stranded RNA, the actual inducer.

How did it work?

The next paper was immodestly called 'Mode of action of interferon'. It seems incredible now that we could have thought that the problem was that simply solved. This short, rather complicated paper, showed that pretreatment of cells with interferon, followed by inactivated virus, led to an increased yield of interferon, a phenomenon called 'priming'. This has now been explained by the induction of otherwise rate-limiting transcription factors required to produce interferon messenger RNA. At the time, the best explanation we could produce, though ingenious, was very complicated, and the conclusion of that paper was remarkably dense and strikingly void of any molecular interpretation. It is a comment on how descriptive our understanding of cellular processes was then. In the event, all this was overtaken when others showed that interferon production was inhibited by treatment of virus-infected cells with actinomycin, an inhibitor of DNA-directed RNA synthesis. Since the virus used to induce was resistant to actinomycin, cellular DNA must be involved. That explained the cell specificity of interferon and provided the essential molecular framework for much of the work that followed in the early sixties.

Antiviral drug potential

Virus interference, which we now believed was mediated by interferon, was not virus-specific, so that one virus could interfere with the growth of a number of unrelated viruses. Could interferon be developed as an antiviral antibiotic? The next paper showed that interferon was active against three pox-viruses, vaccinia, cowpox and ectromelia, although herpes simplex appeared to be more resistant. So it really did look as if interferon could be developed as an antiviral antibiotic.

Interest in interferon was growing. Alick and I wrote an article titled 'Interferon: a possible check to virus infections' for the *New Scientist* in June 1958. We were invited to present our results at a Conversazione for the Fellows of the Royal Society in May 1958. We were all dressed up, in dinner jackets, and when we were asked to present our demonstration a second time at an event to which only the 'great and good' were invited, we had to wear white tie and

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tails, which I had to hire. I vividly remember dressing up in our very modest little North London flat, and sitting down with my wife to eat in my splendour, and she complimented me by putting on an evening dress, as we sat at the kitchen table, before I went off to the great event. It was a heady time; I was only 28.

Trials and tribulations

However, problems were beginning to surface. We were puzzled that we got protection against the growth of vaccinia virus in rabbit skin using chick interferon, for David Tyrrell had found that interferon was species-specific and that chick interferon was not active in rabbit cells. So was our result due to traces of UV virus in the interferon? If so, how many other results were due to traces of UV virus? This troubled us, and coincided with criticism from the US, where interferon was being called 'misinterpreton' and several eminent virologists were dismissing the effects as due to traces of virus. Alick was very depressed by this reaction; the first sign of a series of depressive setbacks which dogged him over the next few years. He was off work for a month or two and I spent that time repeating all the initial experiments with interferon which had been treated at pH 2 to destroy any UV virus, so as to be quite sure that the effects we had been observing, and publishing, were due to interferon and not to traces of contaminating virus. To our relief, all the early experiments held up.

Two lines of inquiry dominated our time for the next few years. The first was to see whether interferon could really be developed as an effective antiviral agent in the UK. In the late fifties the penicillin story still grated in Britain; the perception was that a British discovery had been 'handed over' to the Americans during the war and developed by them into an industrial production process which had been patented, on which we were now paying royalties. So the MRC was under pressure to determine if interferon could be developed as an effective antiviral agent in the UK. This resulted in a novel collaboration between the MRC and three major pharmaceutical companies: Glaxo Laboratories, ICI Pharmaceuticals and Burroughs Wellcome, (later the Wellcome Foundation). Set up about 1958, it had the specific aim of making enough interferon to do a clinical trial. I was a member of that committee, and Alick was chairman. The collaboration had its ups and downs, but it did achieve a trial against a vaccinia virus challenge in the upper arm of unvaccinated volunteers in the spring and summer of 1962. The outcome was two-edged: on the one hand, the collaboration had shown that interferon could be used in humans against a virus challenge, but on the other hand, it was not practical to prepare either enough interferon, or to deliver it early enough to be a useful therapeutic. The clinical development of interferon was put on hold for some years, for we could not make enough of it – a problem not solved until the development of large-scale production in human cells by Cantell in Helsinki and by Finter in the UK, and finally by means of gene cloning in the early eighties.

The other line, which was my responsibility, and filled my time until the early sixties was the purification of interferon, but that is quite another story!

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Further reading

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