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From Interference to Interferon: A Brief Historical Introduction [and Discussion]

J. Lindenmann

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From interference to interferon: a brief historical introduction

By J. Lindenmann
Institute for Immunology and Virology, University of Zurich, Gloriastrasse 30,
P.O.B. 8028 *Zurich, Switzerland*

The idea that a substance mediating interference was released from cells upon contact with inactivated influenza A virus emerged from an abortive attempt at showing that interference was initiated by passage of only part of the virus into the cell, from previous knowledge about interference, and from the use of a simple technique allowing easy separation of fluid and tissue.

Forty years is as good a date to celebrate as any. In 1942, Andrewes, while discussing interference between viruses in tissue culture, added the following comment: 'An alternative to the hypothesis of an exhaustion of food-supply would be, of course, the generation within the cell of some poorly diffusible inhibitory substance' (Andrewes 1942).

What were the facts known to Alick Isaacs in 1956? He knew that heat-inactivated influenza virus was capable of interfering with live virus added later. Heat treatment could be carried out in such a way as to inactivate the neuraminidase, and hence digestion of cellular receptors was an unlikely explanation of interference (Isaacs & Edney 1950). It took several hours for interference to become established, and hence physical blockade of receptors was also unlikely (Fazekas de St Groth *et al.* 1952). It therefore seemed as if interference required some sort of abortive infection on the part of the 'inactivated' virus.

Yet another, at that time unpublished, fact was also known to us: heat-inactivated influenza virus attached (presumably irreversibly) to red cells was capable of inducing interference (Mooser & Lindenmann 1957). This observation sparked Alick Isaac's imagination in an entirely different direction: if interference was an abortive infection, and if virus particles whose outer envelopes were welded to red cells could induce interference, then something must pass from within the virus particle into the host tissue. According to then prevalent views inspired by work with bacteriophages, this 'something' was probably the nucleic acid core of the virus. Alick Isaacs thought that it should be possible to give a clearcut morphological demonstration of this passage of viral core material by looking at 'full' particles before and at 'empty' particles after they had been allowed to induce interference.

Technically, the experiment was to be done as follows. Virus would be inactivated by heat and then attached to erythrocyte ghosts. Ghosts coated with virus would be observed in the electron microscope to reveal normal virus morphology. The same ghosts would then be used to induce interference in fragments of chorioallantoic membrane. After allowing due time for this to take place, the ghosts would again be checked in the electron microscope. It was hoped that they now would look different: hollow shells emptied of their contents.

The most demanding part of this experiment was the electron microscopy. Fortunately an outstanding electron microscopist was willing to help: Robin C. Valentine. His main contribution was to convince us after not too many attempts that the task was hopeless; to drop a project is often more difficult than to doggedly pursue it.

While this work was in progress we approached the same question from another angle: if indeed the virus emptied its core into the host cell, and if this was the event triggering interference, then its interfering capacity should eventually become exhausted. So at the same time that we looked for evidence of morphological changes we also looked for functional deterioration. The interfering capacity should have declined for at least two reasons: hypothetically, because hollow viral shells should no longer be able to induce interference; from previous experience, because heat-inactivated virus kept at 37 °C (the temperature that we used) slowly lost its interfering power. Strangely, the interfering capacity seemed inexhaustible. This fact could best be explained by assuming the generation of new interfering capacity.

The system as used by us contained three elements: the heat-inactivated virus, the erythrocyte ghosts, and the membrane fragment. Had we proceeded systematically, this is the sort of argument we might have developed: we assume that when all three elements are present, new interfering activity is being generated. It would be a lot easier if only two elements were sufficient. So let us try two at a time: virus + ghosts, ghosts + membranes, membranes + virus, and see what happens. In fact, we did not proceed systematically, but according to intuition or to preconceived ideas: since the erythrocyte ghosts had always been considered to play only a passive role, namely as carriers of the virus and as presenting dishes for electron microscopy, the two combinations containing erythrocytes were never seriously considered. Instead, an experiment was set up exactly according to the previous scheme but containing only heat-inactivated virus and membrane fragments. After 24 h, the membrane fragments were removed from the fluid, tested separately to make sure that interference had occurred, and new membranes added to the remaining fluid. This should, for the same two reasons given above, have shown diminished interfering power; in fact it was still highly active, suggesting again that additional interfering activity had been generated (Isaacs & Lindenmann 1957).

At this point the knowledge that interference took several hours to become established probably played an important role. Might not this lapse of time precisely be required for the formation of a new substance? The experiment was therefore repeated under slightly different conditions: contact between virus and membrane was interrupted after a couple of hours, and the membranes were washed and transferred to fresh medium, thereby getting rid of input virus. If new interfering activity was now generated by the membranes, this should readily be detectable, whereas in the previous experiment it could only be estimated against a background of unknown magnitude caused by residual virus. The system of membrane fragments that we used (Tamm *et al.* 1953; Horvath 1954), although not sophisticated even by the standards of the day, was admirably suited to manipulations that required several washings and transfers. In fact, all the technical motions needed for the new experiment had already been exercised in the preceding part of the work.

To our delight, the fluids in which membranes had been floating after the input virus had been removed did contain interfering activity. What remained to be done was obvious: we had to show that the new activity could be differentiated from the virus used to induce it, and to describe some of its properties (Isaacs *et al.* 1957; Lindenmann *et al.* 1957).

Workers who entered the field later have wondered how fast the new idea was received and whether there was a lag between the early phase and later development. When the numbers of papers with the word 'interferon' in their titles appearing each year are plotted, a picture of steady growth emerges (I. H. Sher, personal communication 1982). The data base from which this graph is drawn starts only in 1965. It predicts the dismal figure of 1000 papers to be pub-

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lished in 1982 (see figure 1). Extrapolating backwards to the year 1957 does not lead to the three papers actually written, but to something more like 40 papers. This illustrates two facts. First, as I have tried to explain, the work was firmly based on a large body of continuing research; second, there is no room for any substantial lag period between 1957 and 1965, when 107 papers with 'interferon' in their title appeared.

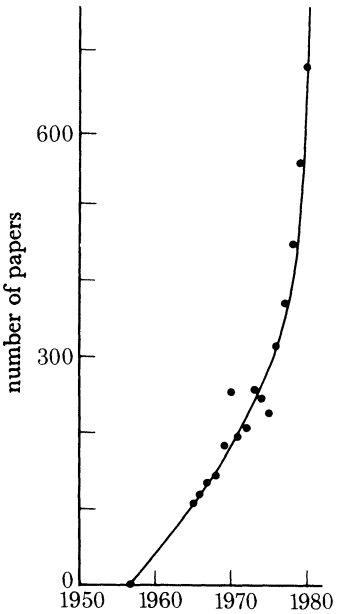

FIGURE 1. Numbers of papers published each year with the word 'interferon' in their titles (data base of the Institute for Scientific Information). (I. H. Sher (personal communication 1982).)

In giving my account of past events I have of course simplified. The threads of thought that I have neatly separated were in reality intertwined, and were criss-crossed by other threads I have either forgotten or not felt worth mentioning. Alick Isaacs might have given a slightly different picture of the same events. All those who knew him will deeply regret that this talk was not delivered by him.

It remains for me to thank the Royal Society for inviting me today; the Medical Research Council, the National Institute for Medical Research, and Sir Christopher Andrewes for accepting me as a post-doctoral worker in 1956; and the Swiss Academy for Medical Sciences for supporting me to the tune of – and this will now really show you how far we have come over the past 25 years – to the tune of £5 a week, which was in fact quite generous.

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Discussion

In reply to a question Dr Lindenmann said that the name interferon was coined as a convenient laboratory shorthand and not as a result of a deliberate taxonomic exercise.

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