Part I: Reflections

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How to Chase a Red Herring and Come up with a Smallmouth Bass

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Abstract The collaboration between Alick Isaacs and myself started in the summer of 1956. Our initial project was to show, by electron microscopy, that interference between inactivated influenza virus and live virus involved the transfer of material from the interfering virus to the host cell. This approach failed for technical reasons. However, in the course of this work it appeared that more interfering activity remained in the system than we were entitled to expect. One possible explanation was that a substance, not identical with the initial interfering virus, was being generated. Subsequent experiments, aimed at checking this hypothesis, led to the description of interferon.

Suppose we were living in a world where every scientific project is a success and yields exactly the results expected. This would be terribly boring, and for my part, I would have chosen a different career. Fortunately, most projects fail, which of course is not very satisfactory either. But occasionally a project, pursued more or less energetically, opens a vista onto a side issue, irresistibly seductive and dangerous. Dangerous, because being seduced is always dangerous, as attested by many novels and operas.

The project, when the collaboration between Alick Isaacs (1921–1967) and me started in midsummer 1956, was the following: we knew that influenza virus particles, inactivated by heat and irreversibly attached to red cells, were capable of inducing interference against challenge with live influenza virus in chick embryos. I had finished this work before I reached Mill Hill in July 1956, but it was still unpublished (Mooser and Lindenmann 1957). Alick thought that we could use this technique to ask the following question: how do the influenza virus particles, with one of their sides firmly attached to the carrier red cells, leaving only their opposite side free to interact with the host cells, induce interference? One possibility was that something was being transferred from the virus to the host. What could that be? From bacteriophage work, it was known that the phages inject their nucleic acid into the host bacterium, the rest of the phage remaining outside. If something similar went on in the induction of interference, one might have a chance to observe this. Before inducing interference the virus particles would be inflated by their nucleoprotein content,

but after having induced interference they ought to be empty, to have collapsed. Their fixation on red cells might offer a way to observe this.

This idea, by the way, is an illustration of an interpretation I have offered elsewhere (Lindenmann 1999): scientists do not do experiments in order to answer the most important questions, but they only ask those questions that suggest possible experiments.

Because Alick Isaacs had already worked with R.C. Valentine (1928–1968), an excellent electron microscopist, we were hoping to be able to document this sequence of events by electron microscopy. We would have a first look at virus particles immediately after their fixation to red cells, before any interaction with host cells had taken place. Then the virus-coated red cells would be allowed to interact with host cells, thereby inducing interference. After that, a second look with the electron microscope would reveal, hopefully, collapsed, empty virus particles still hanging on the red cell carriers.

So this was the project, but to realize it we had to modify the technique I had used, which had been to stick the inactivated virus to intact red cells and do the interference experiment in entire embryonated eggs. Electron microscopy required that we use red cell ghosts, transparent to the electron beam, instead of erythrocytes full of hemoglobin. Furthermore, a very simple technique, based on the use of chorioallantoic membrane fragments from embryonated eggs rather than the entire eggs, was envisaged. This allowed measuring six to eight experimental points (virus hemagglutinin titers) per egg, instead of just one point, thus realizing an economy of material and money and allowing greater freedom of manipulations.

Our first concern was to see whether, with the modifications mentioned, measurable interference indeed occurred. This proved to be the case: red cell ghosts coated with heat-inactivated influenza virus and brought into contact with chorioallantoic membrane fragments induced a state of interference, as measured by the degree of inhibition suffered by a subsequently applied challenge virus, similar to the effect of the free virus that we included as a control.

Had we been obliged to apply for a grant in order to continue our project, what I have just explained would probably have been the basis of our grant proposal. Such a proposal might have been reviewed by an expert in electron microscopy and one in virology. The virologist would have seen nothing very exciting in the proposal, and the electron microscopist would have foreseen difficulties in interpreting the pictures. Both might have suggested that radioactive labeling of the virus contents would offer better chances of success. This is advice we could not easily have ignored. So we should have embarked on a rather demanding additional technique, and before we could have mastered it my fellowship would have expired—even in the unlikely event of our grant application being immediately answered. But we had the good fortune of not having to apply for a grant. The financing of our project was secure: Isaacs's

salary was paid by the Influenza Centre, mine by a fellowship from the Swiss Academy of Medicine, and the (modest) running expenses were covered by the institute's budget.

The experiments I had done in Zurich in 1955 had one major flaw, which was in fact the reason why my boss, Prof. Hermann Mooser (1891–1971), was still brooding over my results: I had assumed that the inactivated virus remained firmly bound to the red cells during the whole experiment. This was not an unreasonable assumption, but it had not yet been proved. How could we show that the virus indeed remained where we wanted it to stay? With Valentine the following exceedingly simple experiment was done: red cell ghosts were prepared and one-half of them were brought in contact with heat-inactivated virus, then washed; these were the virus-loaded ghosts. Three samples were incubated overnight in roller drums at 37°C (the same conditions used to induce interference, except that the membrane fragments were left out): (a) untreated red cell ghosts; (b) red cell ghosts loaded with virus; (c) a mixture of equal parts of a and b. From these three samples electron microscopic grids were prepared. In due time, the pictures were ready to be analyzed and revealed the following: as expected, the untreated ghosts (a) showed very clean and neat surfaces free of any particles resembling influenza virus. The ghosts loaded with virus (b) showed, again as expected, numerous typical virus particles distributed over their surface. However, the pictures from c might have led us to abandon the whole project. They showed, side by side, ghosts containing many virus particles (as in b), empty ghosts (as in a), but, in addition, some ghosts which had a few unmistakable virus particles attached. This probably meant that, in collisions between virus-loaded and empty ghosts, some virus particles had changed their place. Obviously, the attachment of the virus to the ghosts was not as irreversible as we had hoped.

Fortunately, the whole electron microscopy took some time before interpretable pictures were laid on Valentine's desk, and in the meantime we had not been idle. We wondered if the interference-inducing capacity of virus-loaded ghosts could become exhausted. So after a first round of interference induction, the membrane fragments were removed and replaced with fresh membranes to see whether they, in turn, would show interference. We had three reasons to expect this second round of interference to be substantially weaker than the first round: Alick knew from previous experiments that heat-inactivated influenza virus held at 37°C lost its interfering capacity after a relatively short time. Further, we reasoned that if interference had been caused by the virus injecting its nucleic acid into the host cells the remaining empty virus particles would lack activity. Finally, Alick knew that the membranes released an inhibitor into the fluid that impeded the interfering activity of heated virus (Isaacs and Edney 1950). However, contrary to our expectation, this second round of interference was very nearly as strong as the first round.

Now here intuition, or perhaps less mysteriously simply recollection enters into the picture. Our common chief, C.H. (later Sir Christopher) Andrewes (1896–1988) had written in 1942 in a paper on interference between live viruses in tissue cultures:

> The most obvious explanation of the phenomenon is probably the correct one—that the virus first upon the scene uses up some essential foodstuff in the cells. 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.
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> C.H. Andrewes 1942

I don't recall that Alick and I specifically discussed this paper, but we probably had it at the back of our minds. What we certainly did discuss was the wish to see if there was some "generation" of an inhibitory substance ("of course," as Andrewes had written), which might be an explanation for the unexpected persistence of interfering activity. In the seminal paper (Isaacs and Lindenmann 1957), we wrote: "In an effort to explain the results of the last experiment the possibility was considered that fresh interfering activity was produced by the membrane" (p 263). In order to discuss this hypothesis we had to give the unknown substance a name. I suggested "interferon," and Alick thought that this was because I was jealous of my colleagues in experimental physics who were playing with things like electrons, myons, neutrons, baryons, mesons etc.

Until then, our project had involved three elements: (1) the red cell ghost, (2) inactivated virus, and (3) membrane fragments. It now dawned upon us that, under our new tentative hypothesis, the red cell ghosts, always meant to passively carry the virus, could be dispensed with—forget the ghosts. So an experiment was started in which the heat-inactivated virus was brought in contact with the chick membrane fragments for 2 h (bath no. 1). The membranes were then washed free of virus and incubated with fresh fluid (bath no. 2). After having spent a number of hours in this fresh fluid, the membranes were removed and placed in a third bath of fresh fluid (bath no. 3) and challenged with live influenza virus to see if they showed the phenomenon of interference—they did. So far there is nothing new in this experiment. However, now comes the justification for Alick's labeling of this experiment "in search of an interferon" (November 6th, 1956; from Alick's lab journal now kept at the National Library of Medicine in Bethesda, MD): into bath no. 2 a fresh, naive set of membranes was placed, left therein for several hours and then challenged with live influenza virus. To our delight (because by that time we had already become partial, which is a dangerous moment in any investigation), these membranes, which had, as far as we could tell, never been in direct contact with virus, showed clear-cut interference.

The next, rather hectic, few weeks were mainly concerned with the elimination of possible artifacts or of trivial explanations. To give an example of one possible trivial explanation, bath no. 2 could have been depleted of, to use again Andrewes's words lurking at the back of our minds, "some essential foodstuff," although our use of inactivated influenza virus made this proposition less likely than in Andrewes's case, who had been using live virus. Or bath no. 2 might have been teeming with virus particles released from the membranes after temporary capture.

What of the electron microscopy? This proved disappointing, although by that time we were, in this respect, beyond disappointment: the electron microscopic pictures of the ghosts taken after they had been in contact with the membrane fragments could not be interpreted, because they were obscured by cellular debris, so that a distinction between "full" and "empty" virus particles was impossible—the red herring.

I presented some of our results, those involving the red cells, at a meeting of the Swiss Society for Microbiology in Interlaken on June 22, 1957 (Lindenmann and Isaacs 1957). By that time, our two papers in the Proceedings of the Royal Society were still in print, so that this Interlaken meeting was the first official emergence of interferon.

The first metaphor I have used in the title of this paper, the chasing of a red herring (which can be defined as "to follow a distracting clue"), means that we embarked upon an experiment which seemed doable but met with unexpected difficulties. In my second metaphor, the smallmouth bass is described as a game fish which gives the angler a good fight and jumps spectacularly up and down—and I don't have to tell those in the interferon business how many ups and downs they have been through over the past 50 years.

References

Andrewes CH (1942) Interference by one virus with the growth of another in tissue culture. Bri J Exp Pathol 23:214–220

Isaacs A, Edney M (1950) Interference between inactive and active influenza viruses in the chick embryo. II. The site of interference. Aust J Exp Biol Med Sci 28:231–238

Isaacs A, Lindenmann J (1957) Virus interference. I. The interferon. Proc R Soc Lond B 147:258–267

Lindenmann J (1999) Forty years of interferon (preface). In: Lindemann J, Schleuning W-D (eds) Interferon: the dawn of recombinant protein drugs. Springer, Berlin Heidelberg New York

Lindenmann J, Isaacs A (1957) Versuche über Virus-Interferenz. Schweiz Zeitschrift Allgemeine Pathol Bakteriol 20:640–646

Mooser H, Lindenmann J (1957) Homologe Interferenz durch hitzeinaktiviertes, an Erythrozyten adsorbiertes Influenza-B-Virus. Experientia 13:147

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