CONFERENCE LECTURE

INTERFERON: A ROUND UNVARNISH'D TALE

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IT is a great honour to be asked to lecture to the British Pharmaceutical Conference and a particular honour to be invited to give the first of a series of annual lectures. Faced with the difficulty of finding words of my own in which to thank you adequately I have taken refuge in Shakespeare:

> "Yet by your gratious patience
> I will a round unvarnish'd tale deliver . . .
> . . . what drugs, what charms,
> What conjuration and what mighty magic,
> For such proceeding I am charg'd withal."

Research on interferon represents an exciting example of how investigation in what appears to be a wholly academic field of research can lead to more practical prospects. In the laboratory it has been known certainly for 25 years that when cells are infected with one virus they acquire resistance to infection with other unrelated viruses. This is the phenomenon known as virus interference. Scientists were curious about the nature of this cellular resistance and indeed virus interference encouraged research from workers connected with the viruses of plant, bacterial and animal cells. Once the phenomenon had been defined, an important step forward came when it was demonstrated first with bacterial (Delbruck and Luria, 1942) and later with animal viruses (Henle and Henle, 1943) that a virus which had been killed by treatments such as heat or ultra-violet irradiation could lose the power of multiplying in cells but retain the ability to interfere with the growth of other viruses. This gave an opportunity of clarifying the problem. One could then pose questions such as: does the killed virus block the entry of further viruses into the cell, and does the killed virus block an early stage or a late stage in the virus multiplication cycle? This and related topics were investigated in a number of laboratories and it was shown that the killed virus did not prevent the entry of living virus and also that the interference probably occurred at an early stage of the virus growth cycle, that is to say, not only was virus formation prevented but the formation of the building blocks that go to make up the mature virus particles was also inhibited.

The next stage in work on viral interference came in 1957 with the discovery of interferon (Isaacs and Lindenmann, 1957). It was shown that when cells were treated with killed influenza virus a viral inhibitory substance was produced which could be separated from the killed virus, isolated and shown to confer resistance on fresh cells. Essentially this

work involved at first an explanation of the phenomenon of virus interference; we were not consciously searching for an antiviral substance and this only appeared as a later development in the work. At an early stage of this work we made a guess that interferon production represented an abortive attempt of the cell to synthesise virus, but with further investigation it became clear that interferon was completely different from the virus used to induce its production. We now think of interferon rather as a response of the cells to the stimulus of virus infection.

The next question is: what kind of cells respond by producing interferon and what kinds of virus can be used to initiate this response? At the moment we don't know whether viral interference in plant and in bacterial viruses can be explained by the production of a substance similar to interferon. All we can say is that this seems to be rather a general response of those vertebrate cells that have been investigated. Cells of chickens, ducks, mice, hamsters, rabbits, ferrets, cattle, pigs, dogs, monkeys and man have been shown to produce interferon and to be sensitive to its antiviral action.

So far all the animal viruses that we have tested have been shown to initiate the production of interferon, although the actual amount induced by different viruses varies considerably. The viruses tested include small viruses such as poliomyelitis and encephalitis, medium sized viruses such as influenza and mumps, and large viruses such as those of the pox group. Tumour viruses also induce production of interferon. Viruses can be used both live and inactivated, but since any preparation of live virus is not homogeneous and since multiplication can be initiated by only a small proportion of particles in the virus population it is difficult to say which particles are responsible for initiating interferon production. It seems unlikely from what is known at present that interferon is produced by cells at the same time as they are actively engaged in synthesizing virus. It is more likely that the cell can respond to infection in one of two ways—by producing interferon, which then gives it protection against virus multiplication, or alternatively by synthesizing virus. It should be important to try to understand which factors govern the particular pathway that the cell will follow on infection with a virus particle.

It looks, therefore, as if interferon production can be thought of as a cellular response to infection. An early finding in this work was that interferon is liberated spontaneously from cells. It therefore has the possibility of entering the surrounding cells and protecting them. In other words, interferon may not only protect the cell that produces it but the organism itself. This raises the question of whether interferon plays some role in our defences against virus infection. We can consider resistance against virus infection under two headings, firstly recovery from a first infection, and secondly prevention of re-infection. The success of virus vaccines is compelling evidence of the importance of antibody in our ability to resist re-infections. What is not so clear is whether antibody plays anything like such an important role in our ability to recover from primary infections. Doubts about the importance of antibody spring from the fact that recovery from many virus infections

seems to occur at a time before antibody is particularly evident. Also, the experimentalist in the laboratory is continually faced with examples of cells recovering from virus infection *in vitro* under conditions where antibody production does not occur. It is clear, therefore, that other mechanisms must play a role in the recovery processes. It was natural to wonder whether interferon could be important in this respect and careful investigation of tissue cultures chronically infected with a number of different viruses has shown that it is possible for virus and cells to learn to live together in peace over long periods of time. The resistance of these chronically infected cells appears to be due to production of interferon in the cultures (Ho and Enders, 1959; Henle, Henle, Deinhardt, and Bergs, 1959). The same problem can be studied in chick embryos which have been found to show varying resistance to viral infection at different ages. Chick embryos of less than 7 days old show a much lower survival rate after infection with many different viruses than do chick embryos of more than 8 days old. The time at which change in susceptibility occurs appears to be critical and it is closely correlated with the time at which the tissues of the growing embryo develop sensitivity to the antiviral action of interferon (Baron and Isaacs, 1961). These findings suggest that the ability of chick embryos to survive virus infections is closely linked to their ability to produce interferon, to which their cells are sensitive.

Two substances have been found to inhibit the antiviral action of interferon. They are oxygen at high concentrations (Isaacs, Porterfield and Baron, 1961) and cortisone (Kilbourne, Smart and Pokorny, 1961). It was striking to find that increased oxygen tension and cortisone both have a detrimental effect on the course of virus infections. In the case of increased oxygen tension this was shown by experimental infection of mice with influenza virus. Animals kept under increased oxygen tension died more rapidly than animals kept in air (Sawicki, Baron, and Isaacs, 1961). In the case of cortisone this is a clinical observation which has been known for the last few years. It is recognised that patients under treatment with cortisone are at special risk from infection with chicken pox virus. These findings again support the idea that interferon plays an important role in recovery from virus infections.

Other factors too are clearly involved in recovery from virus infection. It is known that high tempertaures inhibit the growth of a number of viruses and Lwoff (1959) has suggested that fever may play an important role in helping in recovery from virus infections. Again, many viruses do not develop well at a low pH, and an inflammatory exudate of low pH may therefore help to play a defensive role. One wonders if these different mechanisms are unconnected or if they may all be linked together. It is possible that raised temperature and lowered pH may act by stimulating the production or the action of interferon. These suggestions will soon be tested experimentally.

Interferon is a protein of molecular weight 63,000 (Burke, 1961) and it acts by protecting cells against virus infection. It has no direct action on virus outside the cells. We have recently found that interferon can

be added to cells in high concentration without inhibiting to any great extent the growth and multiplication of the cells. Such treated cells are, however, highly resistant to virus growth. This suggests that interferon must act by inhibiting the synthesis of viral nucleic acid or protein without significantly inhibiting the production of nucleic acid or protein required in the economy of normal cells. The mode of action of interferon must therefore be quite a subtle one. It is possible that the production of "foreign" nucleic acids or "foreign" proteins is subjected to different control mechanisms from those that control the production of normal cell nucleic acids and proteins and that interferon can block the former without affecting the latter. There is indirect evidence that interferon may act on an oxidative mechanism which is required for producing energy for viral synthesis. At the moment, however, there is no direct evidence on this point, nor is it clear how an oxidative mechanism can be more required in the production of a foreign nucleic acid or protein than in the production of normal cell nucleic acid or protein. This subject will therefore require much further investigation.

Another field which will require investigation is the function of interferon in the normal economy of the body. It seems unlikely that a general property of cells, that is, their ability to produce interferon when stimulated with a large variety of different viruses, should have developed in the course of evolution solely as an antiviral defence mechanism. It seems much more likely that interferon plays another role in the normal economy of the body but that relatively recently in evolution this mechanism should have become adapted to deal with viral infections. One would imagine, therefore, that interferon might play a role in normal cells in controlling the synthesis of nucleic acid or protein of an unusual kind. In speculating about this process one is struck by the fact that interferon shows a very weak antiviral action in the cells of very young embryos and in cancer cells. The implication is that this hypothetical synthesis of a "foreign" nucleic acid or protein may play a role in the processes concerned with differentiation in the very young embryo and that this does not occur in the fully differentiated cells of the normal adult; cancer cells might then be considered as cells which have escaped from this controlling mechanism. These speculations are supported by very little evidence but they have the merit of suggesting a number of experiments.

Interferon has clearly many theoretical points of interest surrounding its mode of action but it has too a practical interest. Laboratory experiments show that it has an antiviral action which extends to a very wide range of animal viruses and that it can be given to cells in very large doses without apparently causing any significant toxic effect. It does seem too that if interferon plays a normal role in recovery from virus infections that the attempt to use it as an antiviral agent in man would be a logical attempt to improve on a natural mechanism of recovery. This is the reasoning which has prompted the Medical Research Council to set up a collaboration with three pharmaceutical firms and it is hoped that in the near future this collaboration will have reached the stage when it should be possible to test out interferon in man. Experiments in animals have

been encouraging and we are hopeful that preliminary experiments may at least repeat a pattern found in experiments in animals. But even if this works out well it is only a first stage in a long investigation required to improve the yields of interferon over those obtainable at the moment, to learn if possible to increase its antiviral effect and to learn how best to use it in the treatment of virus infections in man. The success of such a venture depends on our ability to collaborate in an interesting investigation and one hopes that this partnership between the Medical Research Council and the British pharmaceutical industry will be extended in the future to many other fields of investigation.

We began this work with the investigation of an odd phenomenon, viral interference, and the research has taken us on an interesting journey. Some parts of the road are quite well mapped out already, while for others we will have to retrace our steps and investigate the pathway once more. Yet the glimpses we have of the road ahead make us eager to press on with the journey. The destination is the understanding of what interferon is and how it acts. The closer we can reach to this destination the more rationally shall we be able to use interferon in the treatment of virus infections of man.

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