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ANTIVIRAL ACTION OF INTERFERON*

BY

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Il faut qu'une porte soit ouverte ou fermée.
ALFRED DE MUSSET.

Archaeologists tell us that when primitive man first made tools he fashioned implements that could be used for many different purposes. Interferon is a relatively new tool for the virologist, who may be tempted to use it as an all-purpose implement. I have not myself been successful at resisting the temptation, and this is my apology for trying to see whether interferon can be used to dig a little more deeply into the problems concerned with virus virulence.

Work on interferon began as an investigation of the phenomenon of virus interference (Isaacs and Lindenmann, 1957), in which one virus, once it has infected cells, is able to prevent other viruses from establishing infection in these cells. But when it became clear that interferon production was a reaction of cells from many different species of vertebrates to infection with a large number of different viruses, it could be thought of as a defensive response of cells to virus infection and as a possible factor influencing the recovery process. This possibility has been investigated with cells infected *in vitro* and with chick embryos and experimental animals infected *in vivo*.

In cells chronically infected with virus *in vitro* it has been shown that interferon is produced in the cultures (Ho and Enders, 1959; Henle *et al.*, 1959). It now seems that interferon is responsible for the cellular resistance to virus destruction shown by these cultures. Furthermore, if the interferon is allowed to accumulate in the cultures, complete cure of the virus infection can occur (Glasgow and Habel, 1962). Very young chick embryos are much more sensitive to the lethal action of a number of viruses than older chick embryos. The development of resistance to virus infection in the embryo was found to correspond closely in time to the development of the interferon mechanism (Baron and Isaacs, 1961). In animals which make antibody as well as interferon the situation is more difficult to analyse. Nevertheless, the evidence again favours the view that interferon plays an important part in recovery from virus infections.

Dr. André Lwoff and his colleagues in Paris have investigated another factor important in recovery from virus infections. They have shown that the course of a virus infection can be greatly influenced both *in vitro* and *in vivo* by a small rise in temperature, and they suggest that fever may play a part in recovery from virus infection (Lwoff and Lwoff, 1960). It was interesting, therefore, to see whether the interferon and temperature mechanisms were independent or were in some way related.

*This is a shortened version of an Almroth Wright Lecture given at the Wright-Fleming Institute on May 21, 1962.

Virus Virulence, Ability to Grow at Different Temperatures, and Sensitivity to Interferon

One feature which made this investigation particularly interesting is the fact that it has been found for polioviruses (Dubes and Wenner, 1957) and also for pox viruses (Bedson and Dumbell, 1961) that strains able to grow at higher temperatures are often more virulent than viruses unable to do so. It was possible, therefore, to consider whether virus virulence might be an expression of the insensitivity of a virus to interferon.

My colleague Dr. J. Ruiz-Gomez and I have investigated the growth of a number of different viruses in chick cells over a range of temperatures from 25 to 42° C. Some viruses showed optimal growth at 35°, others at higher temperatures up to 42° C. It was striking to find that there was a close correspondence between the optimal temperature for growth and the sensitivity of a virus to interferon. Among the viruses we studied, the higher the optimal temperature the less sensitive was the virus to interferon. Virulence of viruses for the chick embryo depends, of course, on the age of the embryo, but when these viruses were studied in 10-day chick embryos the most virulent viruses were those that were least sensitive to interferon.

Virus Virulence, Ability to Grow at Different Temperatures, and Production of Interferon

Enders (1960) pointed out that an avirulent strain of measles virus induced human amnion cells to produce higher yields of interferon than a virulent strain, and similar observations were made by De Maeyer and Enders (1962) with regard to polioviruses. Enders speculated that a relationship of this kind might be quite a general one and that it could afford an important clue to the nature of virus virulence. If this were true viruses with a high optimal temperature for growth should give poorer yields of interferon than those with a lower optimal temperature.

When Ruiz-Gomez and I recently investigated this question this is just the result that we found. It is somewhat more difficult technically to compare interferon production by different strains than to compare their sensitivity to the antiviral action of interferon, but it seems clear that the virulent strains, with high optimal temperatures for virus growth, give very poor yields of interferon, whereas the avirulent strains give much better yields. Also the avirulent strains, which grow

less well at temperatures of 37° C. and higher, give their best yields of interferon at the higher temperatures. It looks, therefore, as if the beneficial effect of fever in virus infections that Dr. Lwoff has postulated could act by favouring the production of interferon.

The suggestion that raising the temperature may favour the production of interferon at least for an avirulent strain, and indeed the different behaviour of virulent and avirulent strains, leads to the formulation of a hypothesis which has proved to be very productive. The hypothesis is that shortly after a virus particle enters a cell one of two things can happen. Either the virus stimulates the production of interferon which prevents it from multiplying or it has the reverse effect which allows it to multiply. On this hypothesis an avirulent virus would differ from a virulent virus in having a high proportion of its population made up of virus particles that stimulate cells to make interferon. A population of virus particles of this kind may also be very sensitive to the antiviral action of interferon.

In more literary terms we can describe this hypothesis by saying that the door is either open or closed. The door is in this case presumably the door to a chamber in the cell where virus nucleic acid is synthesized, since we know from the work of De Somer, Prinzie, Denys, and Schonne (1962) that interferon inhibits the replication of viral nucleic acid. The production of interferon is the cell's way of closing this door. A virulent virus is one that can force the door open. Let us see then how the virulent virus acts.

"No Door Can Keep Them Out"—Herbert

The action of a virulent virus could be due to one of two different mechanisms. It could either avoid the interferon mechanism and thus not stimulate the cell to produce interferon or, alternatively, it could actively inhibit the production of interferon by the cell. My colleagues Drs. Z. Rotem and J. Ruiz-Gomez and I have looked to see whether infection of cells with a virulent virus (Newcastle disease virus) would block interferon production by an avirulent virus (Chikungunya virus), and have found that this did occur. This recalls the phenomenon of "inverse interference" described by Lindenmann (1960) in which a strain of live influenza virus inhibited the production of interferon by heat-killed influenza virus. However, we have found that inverse interference is not produced by all strains of influenza and related viruses. Virulent viruses like those of Newcastle disease and fowl plague which grow well in chick cells are able to inhibit the production of interferon by Chikungunya virus, whereas strains of influenza virus that grow poorly in these cells do not inhibit production of interferon by Chikungunya virus. Also, the fact that the virulent strains grow better at higher temperatures is mirrored in the finding that they are better able to inhibit production of interferon at higher temperatures. To return to our metaphor, the virulent virus is able to throw open the door and let the avirulent viruses enter the chamber where their nucleic acid is replicated.

In speaking of virulence we have perhaps taken a rather one-sided view. Virulence is, of course, not a property of the virus alone but of the virus in relation to a particular cell. This is shown most strikingly by Newcastle disease virus, which is virulent for chick embryos, grows well in chick cells, and inhibits interferon production, whereas the same virus does not grow in cultures of human thyroid cells, but, on the contrary, it stimulates these cells to produce interferon in high titre. In this case we would talk of thyroid cells as being insusceptible, as Shakespeare speaks of men that "shut their door against a setting sun." The chick cells resemble rather Robert Browning's sportive ladies who "leave their doors ajar."

Factors that Influence Operation of the Interferon Mechanism

We have already seen three factors that decide whether a virus-cell interaction will result in virus multiplication or interferon production. This first is the virus, or we might say the virus virulence; the second is the cell; and the third is the temperature—raising the temperature favours interferon production with an avirulent strain but it favours the blocking action with a virulent strain. A fourth factor is the presence of interferon itself. We have earlier found that cells treated with interferon respond to virus infection by producing more interferon rather than producing virus (Isaacs and Burke, 1958). In the present experiments essentially the same results were found. Interferon-treated cells were unable to support the growth of Chikungunya virus but were able to produce their full yield of interferon. Previous treatment of cells with interferon therefore makes it more probable that a virus–cell interaction will result in the production of interferon rather than virus multiplication. This would, of course, provide a very convenient explanation for the fact that viruses that are good interferon producers are also very sensitive to the antiviral action of interferon.

There is suggestive evidence from other work that interferon may act on an oxidative mechanism in the cell (Isaacs, Porterfield, and Baron, 1961). We therefore investigated the action of lowered oxygen tension on cells infected with Chikungunya virus. We found that cells incubated in an atmosphere of nitrogen behaved just like interferon-treated cells—that is, virus multiplication was completely inhibited whereas production of interferon was not affected. Oxygen tension is therefore a fifth factor that influences whether virus multiplication or interferon production will occur.

A sixth factor is *p*H. Lwoff and Lwoff (1960) had found that lowering the *p*H to about 6.8 inhibited the multiplication of certain polioviruses just as raising the temperature did. Recently De Maeyer and De Somer (1962) have found that at *p*H 6.8 cells infected with Sindbis virus produce more interferon than at *p*H 7.4. The cells are also more sensitive to the antiviral action of interferon at *p*H 6.8.

We know also that treating particles of some viruses with ultra-violet light changes them from a population able to block interferon production into producers of interferon. Presumably other factors will be found that will decide whether the results of a virus–cell interaction lead to production of interferon or virus multiplication. In passing, it is noteworthy that there are other situations in which the room temperature and the degree of aeration may help to decide whether the door is open or closed.

Speculation

Elsewhere I have suggested that since interferon production is such a general reaction of cells to infection with so many different kinds of viruses, perhaps it is essentially a reaction of cells to a foreign nucleic acid, by analogy with antibody production, which could be loosely described as a reaction of the body to a foreign

protein. If we pursue this suggestion, the process of virus adaptation to a host which involves an increase of virus virulence and a decrease in its ability to excite the production of interferon might in some way involve some subtle change in the viral nucleic acid by means of which it came to seem less foreign to its new host. This may be the way in which a virus acquires a key to the door of the cell's nucleic-acid-synthesizing chamber. It is our job to learn the combination and to find how to prevent it from being forced.

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UREA DISTRIBUTION IN THE BODY AFTER HAEMODIALYSIS

BY

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With increasing use of the artificial kidney as a definitive method of treatment in some cases of renal failure, opportunities have arisen to study the effects of haemodialysis on urea distribution in the body. Although it has been generally accepted that urea is freely and rapidly diffusible in the body fluids of normal subjects (McCance and Widdowson, 1951), the validity of this concept in uraemic patients undergoing haemodialysis on an artificial kidney has recently been questioned, and evidence has been adduced to support a contention that a relatively non-freely diffusible intracellular urea exists in such circumstances (Blackmore and Elder, 1961).

In individual uncomplicated cases of acute oliguric renal failure the pre-dialysis daily increment of plasma urea concentration is virtually constant: indeed, practical use is made of this phenomenon to anticipate and plan the day for haemodialysis (Loughridge *et al.*, 1960). After haemodialysis the daily increment of plasma urea concentration is virtually identical except for the first day, when there is almost invariably an accelerated rate of rise (Fig. 1). There seems little reason to believe that this latter phenomenon reflects a temporary increase of metabolic rate in response to rapid removal of urea by haemodialysis, or that it results from an accelerated production of urea due to the trauma of haemodialysis. An alternative explanation—

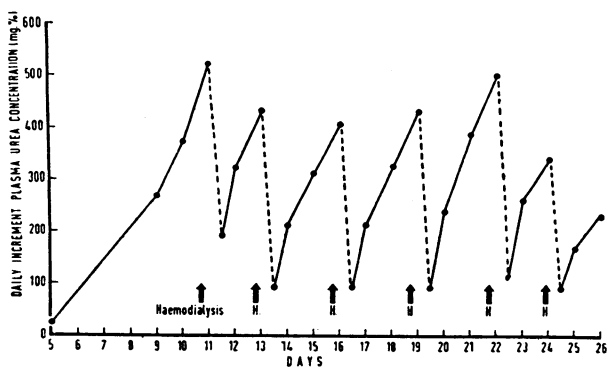

FIG. 1.—Daily increment of plasma urea concentration in a case of acute renal failure treated by haemodialysis on six occasions, showing the accelerated rate of rise during the first day after each haemodialysis.

namely, that the extracellular urea concentration, as measured by the plasma urea, does not reflect, and indeed is less than, the intracellular urea concentration in the immediate post-dialysis period, and that equilibration subsequently takes place in the following day—seems more reasonable. To test the validity of this hypothesis it was decided (1) to determine the intracellular concentration of urea in a series of muscle biopsies taken immediately after haemodialysis and to compare the values with the corresponding plasma values of venous blood samples withdrawn at the time of the biopsies, and (2) to observe post-dialysis hourly rates of rise of the plasma urea concentration and to determine the average time taken for equilibration with the pre-dialysis rates of rise.

Material and Methods

Muscle Biopsies

Intracellular urea concentrations (expressed as mg./100 ml. of muscle water) were determined in muscle biopsies obtained from the medial part of the gastrocnemius muscle in 19 unselected patients with acute renal failure immediately after haemodialysis on a rotating coil artificial kidney. Local anaesthesia (2% lignocaine) was used to infiltrate the overlying skin. Care was taken to avoid fat and connective tissue, and 2–4-g. muscle samples were obtained with dry instruments and were placed immediately into dry glass containers. Venous blood samples for urea, electrolyte, and haematocrit determinations were withdrawn at the time the biopsies were taken and all specimens were transferred immediately to the laboratory. There the muscle samples were blotted to remove surface blood, trimmed of any visible fat and connective tissue, and then divided into two approximately equal portions.

The paired portions were transferred to two dry tared weighing-bottles and accurately weighed (wet weight). One of the paired portions was shredded with fine dry scissors and then crushed with a glass rod. Then 1.5–2 ml. of distilled water was added to the crushed specimen in the weighing-bottle, washing off the glass rod, and the whole was accurately weighed and then put into a refrigerator at 4° C. for 24 hours to allow for equilibration. At the end of this time 0.2 ml. of the