Papers and Originals

Tumour-viruses and Virus-tumours*

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It is an honour to be invited to give this lecture commemorating the late Professor E. H. Kettle. I saw a good deal of him at one time, for he succeeded my father in the chair of pathology here at Barts in 1927. My lecture concerns a field which was one of his major interests. His handbook *Pathology of Tumours* is, in the words of J. A. Murray, "a useful and shrewd summary of the fundamental problems of tumour pathology." Though he did not work on the particular aspect of the problem which I am to discuss, he would, I am sure, have been sympathetic to this approach because of his friendship with W. E. Gye. In thinking of Kettle my mind naturally turns to Gye, with whom I once worked and with whom Kettle was closely associated shortly after the first world war in work on silicosis. Gye later became a most powerful advocate of the idea that cancer could be caused by viruses. It is ironical and sad that though he spent years in trying to prove that viruses were concerned in cancer in mammals, his proffered evidence failed to carry conviction; yet only a few years after his death, with emergence of new techniques, viruses as causes of tumours are being discovered here, there, and everywhere. Gye must always hold a special place in the history of cancer research. Peyton Rous's discovery in 1910 of his fowl-tumour viruses formed a contribution of outstanding importance; yet it was generally ignored. Pathologists said either that the fowl sarcoma was not a true tumour or else that the filterable agent was not a true virus. Gye brought it into daylight again and made people realize that these criticisms had no just basis. He greatly helped to prepare the way for the recent astonishing increase of knowledge about viruses as causes of tumours.

In this lecture I shall be telling you of no original work or original theory of my own, but trying to bring some of the recent work of other investigators into focus.

I chose the title of my talk because I wanted to draw attention to two important questions. First, are there such things as tumour-viruses or, rather, are so-called tumour-viruses any different from other viruses? And second, are virus-tumours any different from any other tumours? Do viruses in fact cause far more cancers than might be suspected? I hope to show you that the first question can be answered rather easily; but the second, not yet.

What Are Tumour-viruses?

Let us consider the place of tumour-viruses in the array of knowledge concerning viruses generally. We have only recently learnt something about how viruses are best classified. We now divide them, first, into those containing ribonucleic acid (R.N.A.) in their make-up, and those containing deoxyribonucleic acid (D.N.A.). All true viruses contain one or the other, not both; bacteria, even rickettsiae and the large so-called viruses causing trachoma and psittacosis, contain both. Next we divide up viruses according to their architecture, whether their protein subunits are built up round their nucleic acid in a cage of cubical symmetry, or whether these subunits are wound round the nucleic acid to form a twisted helical core inside the virus. And then we divide them further into those with outer lipid-containing membranes and those without. Using these criteria, we can group the large majority of viruses into one or other of eight or nine families. It appears that tumour-causing viruses are by no means all closely related. Quite a number of them are included in a family of small D.N.A. viruses with cubical symmetry and no outer membrane, the so-called papovaviruses. Here we find wart-viruses, including some whose growth may become malignant, the polyoma virus of mice, and several others. In another family, the adenoviruses, rather similar but a little larger, are found more tumour-producers. On the other hand are the viruses causing leukaemia in mice and in fowls and one causing mammary cancers in mice; these are R.N.A. viruses with, probably, helical symmetry and an outer envelope. Some of the family of pox viruses also cause tumour-like growths which may in certain circumstances become malignant.

It is apparent that tumours are caused not by viruses with unusual properties but as a result of a particular state of association between cells and viruses of quite different kinds. All sorts of viruses can become oncogenic.

We now have to recognize the existence of an extraordinary anomaly. On the one hand, viruses may cause cancers and yet no longer be demonstrable in the growths for which they are responsible. On the other hand, we may find in tumours all sorts of viruses, some of which may indeed be capable of causing leukaemias, but which nevertheless have nothing to do with the causation of the growths in which they were found. I shall refer later to the tumours caused by polyoma and adenoviruses from which virus has disappeared; but I will say a few words about the obverse side of the anomaly.

In thin sections of virus-infected tissues one can often find collections of similar virus-particles, often referred to as target-like. That is to say, there is visible an electron-dense nucleus-like mass, the nucleoid, with one or more surrounding membranes. Such objects have been found in many cancers, even some human ones, and have been prematurely assumed to be the agents causing those cancers. Or, again, extracts from cancers have been used to infect tissue cultures, and viruses have been grown out and falsely accused of causing the tumours which yielded them. This pitfall is now being avoided. The explanation for the finding is probably this. Besides the known pathogenic viruses there are others which are quite harmless, just as there are many innocuous bacteria. Or else, after a virus disease, some particles may

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survive and persist in the body, normally undetected and perhaps maintaining that permanent immunity which fortunately follows many virus infections. It is believed that a protein, interferon, is made by cells which are attacked by a virus and that this is able to antagonize it and halt its progress by deftly manipulating the cell's metabolism in a way hostile to the virus. In tissue cultures, and probably in the living animal also, virus infection may be slowed down by interferon. It may be wholly got rid of or the result may be a balance of growth between virus and interferon, the virus never being quite eliminated, yet unable to do the cell any serious damage.

It appears that cancer cells do not produce interferon as efficiently as do normal cells, or that interferon does not work so well in them. So a virus which would be disposed of in the ordinary way manages to persist in a cancer. The advantage is tilted just so much in the virus's favour. Then along comes a medical research worker; he finds fascinating virus-like bodies in the tumour, or he exalts the virus still further by cultivating the tissue *in vitro* or by serial passages in fresh hosts; and he ends up with an agent which greatly excites him. Curiously enough, quite a number of the viruses thus turning up have been obtained from mouse tumours passed serially by cell transplantation and they have themselves produced not solid tumours but leukaemias. Again, serial passage of leukaemias in mice has on several occasions brought to light a virus causing fatal hepatitis. The viruses thus revealed have proved to have great interest in themselves, but from the point of view of discovering the cause of the original tumours they must, I am afraid, be dismissed as red herrings.

Multiple Factors in Causation

We will accordingly turn to some of the viruses which really do cause tumours, and it is convenient to look first, not at the first described one, the Rous sarcoma, but at the virus discovered by Bittner as concerned in mammary cancer of mice. It has been called a milk agent, an inciter, and various other things because of people's reluctance to believe that a virus could cause such an absolutely typical cancer as mammary carcinoma. But it is now admitted to be a perfectly typical virus. It is worth looking at because it admirably illustrates the difficulty of deciding what is the cause of a disease. Would-be matchmakers know all too well that you cannot ensure an engagement and marriage simply by bringing two eligible young persons together. So with a parasite and its host. A successful introduction may be easy or difficult. Measles virus brought into contact with a child causes measles—provided only that the child acquires an adequate dose and has not had measles before. The Bittner virus causes mammary cancer in mice, but the provisions you must specify are far more numerous and elaborate. The virus must be given to the mice when they are very young; normally it is transmitted from the mother in the milk. Then the mice must be of the correct genetic constitution; most studies have been made with inbred mice. Finally, the hormonal environment must be right.

No cancer will follow if one of the conditioning factors is missing, but if they are present at low levels it does not necessarily do so. If one of them is very potent it may lead to cancer even if the others are present only at rather low levels of activity. Coyness in a damsel may be overcome by great ardour in a swain, by a full moon or constant proximity on a bathing-beach. So an optimal dose of Bittner virus will lead to mammary cancer in a relatively resistant strain of mice; or it may follow oestrogen injection into male mice, which are normally free from mammary growths. The result of this sort of thing is that people are apt to conclude that *the* cause of cancer is that one of several contributory causes which is so unusual that it hits you in the eye. If it turns out that viruses are concerned in the causation of many cancers, including human ones, it will be in this sense.

Another illustrative example is afforded by the occurrence of leukaemia in the Ak strain of mice, as described by Ludwig Gross. These mice have a high incidence of leukaemia anyway. Give baby mice a big dose of the virus that can be extracted from such leukaemias and you will have a much higher incidence than normal at an earlier age than normal. Virus appears not so much to cause as to accelerate the occurrence of the disease. X-radiation, as you know, can lead to cancer or leukaemia. Leukaemias induced by radiation have been shown by Gross and by others to yield a leukaemia-causing virus. When Furth and his colleagues (1962) irradiated adults of the RF strain of mice they obtained leukaemias—some myeloid, some lymphoid—in 33%. Gross's leukaemic virus led to 4% of lymphoid leukaemia in such mice. The combination of virus and irradiation was followed by the occurrence of leukaemia in 57%.

Similar findings are revealed by Rous's studies on the combined effects of tar or carcinogenic hydrocarbons and Shope's papilloma virus in rabbits. In earlier experiments Kidd and Rous (1938) described how on the ears of rabbits prepared by tarring, papilloma virus would localize and how the resulting warts would behave abnormally from the start; they would then frequently go on to become malignant and that far more rapidly than without the tar. In later experiments Rogers and Rous (1951) varied the technique. They applied virus and hydrocarbons (20-methylcholanthrene or 9 : 10-dimethyl-1 : 2 dibenzanthracene) simultaneously to rabbits' skins made hyperplastic with turpentine. In the presence of the hydrocarbons the warts actually did rather worse than those in the controls. Nevertheless far more of them became malignant.

All these queer results can be explained if we remember that there are multiple factors in causation and that startling, though at first confusing, things may happen if we enhance any one of them; still more so if two factors are increased at the same time. How all the operative factors work and how the co-operation is effected are difficult matters to discover.

A rather different aspect concerns the lessening of cancer incidence by removing a factor which is normally present and so not immediately obvious as being a factor at all. The outstanding example is the effect of thymectomizing very young mice in preventing or lowering the incidence of leukaemia whether this is spontaneous or set up by injection of a virus. This does not appear to be merely due to removal of the only place where the leukaemia virus can multiply (Miller, 1962). Removal of the thymus also reduces the incidence of lymphomas induced with methylcholanthrene.

Bittner's mouse mammary virus is here of interest. As is well known, virus can be transferred to mice of a low cancer strain by suckling on mothers of a high cancer strain. Cancers may then develop, particularly in females after forced breeding; in the absence of the hormones thus stimulated the females may not get cancer, yet will pass the virus on to their offspring. So on indefinitely if the mice are of a suitable strain. But if the mice are of a resistant strain, reluctantly accepting the virus, its potentialities will become less and less in succeeding generations and it will ultimately become extinguished.

Stages of Carcinogenesis

There is general acceptance now of the idea that carcinogenesis is a two-stage affair. The first stage is that of initiation, whereby some agent acting upon a cell produces in it a change, normally irreversible, endowing it with the potentiality of going ahead to form a cancer. The carcinogenic

agent, perhaps a hydrocarbon or some form of radiation, need play no further part. The cell or cells thus affected need not go on to form a cancer forthwith ; they may remain dormant and only break loose when the second stage, that of promotion, occurs. Promoting agents are less specific than initiators. They may act, as does croton oil, to cause proliferation of cells, particularly those latently cancerous ones, so that they are now obviously malignant. Withdrawal of the promoting agent may lead to temporary or permanent regression of the growth, or the cells may have undergone some change giving them the impetus to go ahead on their own. In the case of mammary cancers, hormones may act as promoting agents and the cancers may or may not be hormone-dependent, able or unable to continue growing in the absence of the hormone. Some cancers caused by the Bittner virus may be hormone-dependent. Some substances probably act both as initiating and as promoting agents. Much of this we know from the work of Peyton Rous.

Where do viruses come in ? When the only known tumour-virus was that of the Rous sarcoma, one could show clear distinctions between things like hydrocarbons, which did their fell deed and then were gone, and those like the chicken-tumour virus, which were present as a readily demonstrable continuing cause. Things, as we shall see, are not so clear-cut nowadays. It still seems reasonable to believe that viruses act as initiators and may or may not be promoters also.

There is the very fascinating question of whether or not tumour-viruses always persist in any form in virus-tumours. When Rous sarcoma is propagated in chickens it may go for several consecutive serial passages through a phase in which no virus is demonstrable in filtration experiments ; it behaves in fact like a non-filterable mammalian tumour. This is more apt to happen when conditions are unfavourable for the virus, as when very small doses of virus are given or when older fowls are inoculated. The difference from an ordinary filterable tumour is, however, only quantitative. Better methods of extraction may reveal the virus. And the tumour's filterability will be restored once more after passage under more favourable conditions. The virus was perhaps being masked before by antibody or by an inhibitor such as interferon.

Persistence of Viruses in Tumours

The rabbit papilloma reveals another state of affairs. In its native host, the American cotton-tail rabbit (Sylvilagus), papillomata are usually full of virus. In domestic rabbits the warts grow well but virus is much less readily demonstrable. Sometimes no active filtrate is obtained and the presence of virus can only be revealed indirectly by the development of antibodies in rabbits injected intraperitoneally with extracts of the warts. There has been dispute on whether the domestic rabbit warts contain virus simply in smaller quantity or in a modified or masked form. Noyes (1959) has shown that virus may be revealed by means of fluorescent antibody mainly in the keratinized layers of the skin, though abnormal cell proliferation and virus-production would be expected mainly in the more active deeper layers. Shope has suggested that it may be here in the form of D.N.A. and that in the tame rabbits the maturation to complete infectious virus fails to occur.

When domestic rabbit warts go on to become malignant, virus is usually demonstrable only indirectly through the antigenicity which persists. In this form viruses may be carried through many transplant generations of carcinoma. In one derived cancer, however, evidence of the virus's presence can no longer be obtained. Either it has wholly gone or it is integrated with the cell very closely and no longer makes antigen in demonstrable quantity.

Polyoma Virus

I have had no occasion until now to do more than mention polyoma. This is a virus which is very widespread and under natural conditions hardly ever causes cancers. Like other viruses which I mentioned earlier, it was discovered through its presence in serially propagated leukaemias. For some time it confused research very much as no one knew whether the parotid tumours and leukaemias which were being produced were due to the leukaemia virus which was the original agent under study. Polyoma virus belongs to the family which I mentioned earlier, the papovaviruses. This name is made up of PA for papilloma, PO for polyoma, and VA for the vacuolating agent I shall mention later. These viruses are medium-sized ones with protein subunits regularly arranged so that the whole thing forms a twenty-sided figure. There is no outer membrane and virus growth is in the nucleus where the virus particles may be tightly packed in a crystalline arrangement or may be more widely scattered. The virus's oncogenic powers are revealed when, first, it is greatly increased quantitatively by growing it in tissue culture, and, second, when it is inoculated into newborn or at least very young animals. In baby mice it leads to parotid tumours, sarcomas, and all sorts of other tumours, often multiple—hence the name " polyoma." In other species—infant rats, hamsters, and even ferrets—it causes chiefly sarcomas.

All sorts of interesting things have developed from the study of polyoma. It is found to initiate tumours in hamsters within a few days. Rous-sarcoma virus also acts within a few days, but some of the other tumour-viruses, the mouse leukaemias and the Bittner virus, show their effects only after weeks or months. Polyoma can transform cells of mouse, rat, and hamster in tissue culture. These transformed cells are thought to be either malignant or to be part way along the road to malignancy. They show abnormal mitoses and they show lack of contact-inhibition. Normal cells are inhibited by social decencies from proliferating rudely when they come up against other cells : malignant cells have no such inhibitions but multiply and scramble madly all over each other. Cells transformed by polyoma also, like malignant cells, grow under harsh cultural conditions where normal cells cannot. They may or may not cause cancers when inoculated into genetically similar hosts. The frequency of such transformations can be studied quantitatively in culture, and information about the process is accruing rapidly.

If one works as is ordinarily done with Rous sarcoma, and filters a polyoma-induced tumour with the idea of passing the tumour on in series with a filtrate, one fails, though transplantation with minced tissue is possible. This is because only a little free virus is extractable from the tumours. Serial passage with filtrates is possible only by alternating tissue culture, which boosts up the virus titre, and mouse inoculation.

It may also happen that one fails to recover virus from the tumours at all ; this is more commonly the case in hamsters. The virus now seems to have acted as a chemical carcinogen may do, starting the cell on a career of malignancy and then disappearing. This finding raises the question of what tumour-viruses are doing anyway, when one finds them in a tumour. We know that non-oncogenic viruses may be found as passengers in a tumour, finding the situation to their liking. Why should not oncogenic ones, tumour-viruses, equally be carried along as passengers, once they have started a train of events ? They may be like a naughty boy who finds a car on a slope and takes the brake off. The end is inevitable whether he remains in the car or not. Perhaps in some instances he stays at the wheel : the Rous virus may be a continuing cause of its sarcomas.

Complete disappearance of polyoma virus from its tumours is by no means certain. Karl Habel (1961) made the remarkable observation that adult mice immunized against polyoma

virus were resistant to transplantation of polyoma-induced tumours. It appeared that the action of the virus had led to the production of a new polyoma-tumour antigen, distinct from any polyoma-virus antigen which had been recognized. He supposed that when cells of adult mice, which are more immunologically competent than baby ones, were infected by polyoma and underwent some transformation, the mice reacted against the new tumour antigen which resulted and so no tumour developed. Then when they were later grafted with polyoma-induced tumours they were able to react against and reject these also.

Simian Virus 40

There is another papovavirus which can produce tumours in baby hamsters. (Hamsters are very remarkable animals in their reaction to many stimuli, but especially to tumour-viruses.) This other virus is the vacuolating agent, simian virus 40, commonly called SV40. Monkey kidney intended for use in making polio vaccine or for other purposes is often contaminated by naturally occurring simian viruses, which have, of course, to be avoided. One such, this SV40, was for long undetected in batches of polio vaccine, for it normally produces no effects in cultures of rhesus kidney and was discovered only when it came to be tested in kidney cultures from African monkeys. Before that it had been unknowingly given to many children with their polio vaccines, and in them it multiplied, though without giving rise to any detectable harmful effects. If, however, it is injected into newborn hamsters it can cause sarcomas, as polyoma does. As with polyoma, too, it seems to lead to production of a new tumour antigen, so that administration of virus to hamsters renders them resistant to transplants of SV40-induced tumours. The antigen is, however, distinct from the one concerned with polyoma. A rather disturbing feature is that this virus will grow in tissue cultures of human buccal mucosa and skin, and in them will induce transformations with chromosomal abnormalities just as polyoma can do in rodent tissue cultures (Koprowski *et al.*, 1962).

A more familiar kind of virus of the adenovirus family can, in baby hamsters, do the same sort of thing. Only 2 of the 28 serological types have so far been incriminated, types 12 and 18, two of the less common ones. These tumours show something of enormous interest. They reveal the same phenomena as with polyoma and SV40 : in them, too, transplantable tumours may develop, yet virus is no longer recoverable. With them also a new tumour-antigen appears, related to immunity against cell transplantation. But this time there is a difference : despite the absence of detectable virus, one can show an immunological relation between the new antigen and a specific serum against the initiating virus. This antigen continues to appear in subsequent transplants (Huebner *et al.*, 1963). Here, then, is evidence sought in vain with the cancers derived from papilloma, from polyoma, and from SV40 that in these cancers is recognizable virus, present but not infectious. Whether it is driver or merely passenger we do not know.

"Toothless" Viruses

This discovery fits in with notions about viruses and tumours which I have held for a long time. I discussed in 1939 whether cancer could be mediated by an intracellular virus which had lost the power of infectiousness. May I quote from what I then wrote? (Andrewes, 1939). Some "findings make one wonder whether a virus may not depend for its power to infect normal cells on some . . . aggressive mechanism, teeth as it were, permitting an entry into the new cell ; and whether in certain circumstances a virus may not lose its teeth by disuse-atrophy. . . . This could happen most readily, one may imagine, in the environment of a cancer-cell, where virus could be carried from cell to daughter and granddaughter cell as cell-division was stimulated and the need to come out of the cell to look for fresh prey would disappear. Viruses which had become toothless by some such process could thus act as a proximate cause for cancer, and yet one would never be able to demonstrate their presence by injecting tumour-filtrates into fresh hosts." I continued : "It is not too easy to reconcile the conception of a toothless virus in a cancer-cell with the need for visualizing some possible means for carrying the virus over from one generation to the next." We have learnt a lot about viruses in the 24 years since I wrote that, and the last point would not worry me to-day. We now believe that all sorts of viruses, infectious in some conventional manner, may insinuate themselves into a cell and set up a state of affairs leading to cancer, perhaps later becoming "toothless" in the process.

Following different clues we are led to two notions as to the role of viruses in cancer. According to one, the virus sets off an inevitable train of events, and if it persists in the cancers which result, that is an irrelevant happening which might equally affect any virus which was around. According to the other view, the tumour-inducing virus is integrated into the cell, losing part of its anatomy in the process, but acting as a continuing cause of cancer by virtue of this integration. Maybe either thing can happen, but it would be tidier if all viruses acted the same way.

Viruses and Human Cancer

All these thoughts necessarily affect our approach to the problem of whether or not viruses cause cancer in man. It would seem unlikely that cancer should have a different cause in different areas of the animal kingdom ; and recent work on mice makes us realize that viruses are important oncogenic agents in this area. Unfortunately the techniques so widely used among mice cannot be applied to human beings. Or can they?

Some recent findings indicate possible approaches. Electron-microscopy, it is true, enables us to recognize more surely than a few years ago which of the bodies we see in thin sections are viruses and which are not. But the presence of the passenger viruses we have discussed makes it impossible to conclude that virus detected in a tumour has caused that tumour, without seeing whether it is oncogenic in man : which, of course, one cannot do. One can, of course, see whether an extract will produce transformation of cells in cultures of human tissues, as the SV40 does. That would give a strong hint but would not settle the matter.

Immunology may help. What we have learnt lately must tell us to take advantage of any hint or clue. We must not expect the villain to look necessarily like a ruffian with a black mask and a jemmy, but perhaps be someone in the guise of a familiar citizen, a citizen who has unfortunately taken to evil ways. There has been great interest lately in malignant lymphomas in tropical Africa. These are extremely rapidly growing tumours which particularly affect the jaws of young children, but may also affect other organs. The remarkable thing about them is that they are particularly prevalent in a belt right across tropical Africa south of the Sahara, but only below a certain altitude, roughly 5,000 ft. (1,525 m.). The distribution is said to correspond with that of man-biting mosquitoes. These facts have led to the dispatch of more than one party to central Africa to look into the matter. I understand that attempts to demonstrate a filterable causative agent have had no great success as yet. I am not surprised. We have learnt that it is no unusual thing to fail to find an infectious virus in a virus-

caused tumour. But the hint, albeit tenuous, that the causative agent may be mosquito-borne may help us. One immediately thinks of the family of arboviruses. This name is a telescoped form of arthropod-borne virus. There are probably 150 viruses in this family already known, with more being discovered every year. Some such as yellow fever cause well-known diseases, others mild fevers. Others again have not been associated with disease, having been isolated from mosquitoes or forest animals; yet antibodies against them have been found in some human sera. Quite a number of these occur in tropical Africa, and serological study is, I understand, being made of the incidence of antibodies against these in people with and without lymphoma.

Then there is the question of the adenoviruses already mentioned. When their oncogenic potentialities were made known there were headlines in the press about common colds and cancer. This need not cause alarm, as adenovirus infections are not like ordinary common colds, and man does not react just like a newborn hamster. Still, the facts should be borne in mind. Early in 1963 I was in Singapore and Hong Kong, and I inquired about the prevalence there of common colds. Though colds, of course, occur, the accounts given me of the most tiresome minor respiratory illnesses reminded me rather of the clinical picture of adenovirus infections. I was also told that one of the commonest kinds of cancer in those parts was a nasopharyngeal carcinoma. The two things may be unrelated, but I should think such a clue was worth following up, particularly since adenovirus tumours in hamsters tend to carry the hall-mark of their origin.

New Facts about Fowl Tumours

I have in this lecture mentioned the Rous sarcoma incidentally. I will say a little more now because of novel things coming to light. I shall not say much, because so much is still uncertain and I am myself confused as to what to think. There appears to be a family of viruses rather closely related, both antigenically and in their general properties. They are fairly large R.N.A. viruses with outer membranes, and apparently have their nucleoprotein in a helical core like an influenza virus. They all attack fowls and may cause sarcomata, visceral leucosis, erythroblastosis, or myeloblastosis; or they may exist as latent viruses in normal birds. When passed in series they normally breed true—that is, sarcoma yields sarcoma and the leucosis viruses the same type of disease—but they do not always do so. They may be rather labile agents, a few strains studied in laboratories having more firmly fixed characters. Dr. Rubin and his colleagues in California have been studying the behaviour particularly of the Rous virus in tissue culture. Amongst other things there were discovered what seemed to be two viruses in normal fowl cells, called the resistance-inducing factor (R.I.F.) or the Rous-associated virus (R.A.V.) (Rubin, 1961; Rubin and Vogt, 1962). The R.I.F. virus seems to be an avirulent form of visceral leucosis virus and has the power under appropriate circumstances of inhibiting the activity of the Rous virus. It appears, however, that in other circumstances the Rous virus cannot manifest its activity without co-operation from one of these additional so-called "helper" viruses (Hanafusa *et al.*, 1963). Some of the information awaits less contradictory interpretation, so I will leave it at that for the present.

The Rous virus was for long only able to infect domestic fowls, and I was very excited, a long time ago, when I found that I could infect pheasants, which are zoologically not very remote. However, that was nothing! Other workers succeeded in infecting turkeys, quails, and ducks. Now a strain of virus has been found causing tumours which can be propagated in series in rats, mice, and hamsters. Professor C. G. Ahlström and his colleagues at Lund achieved this with the Schmidt-Ruppin strain (Ahlström and Jonsson, 1962). Russian workers had previously described production of haemorrhagic cysts in rats, but Ahlström has taken the thing further. The tumours produced are almost certainly formed by infection of rodent cells—it is not a question of survival of fowl cells in the rodents. Tumours can be produced in fowls after many passages through mice and rats, but, oddly enough, filtrates of the rodent growths will not infect birds, though when tumours are reproduced in the birds they can again be transmitted with filtrates quite easily. It may be a quantitative affair, the rodent tumours not containing enough virus, just as polyoma tumours do not readily filter without being enhanced in tissue culture. Or a helper virus may be missing. The latest news about the Schmidt-Ruppin fowl tumour is that it has produced tumours in baby monkeys (Munroe and Windle, 1963).

Unanswered Questions

Two very important questions remain unanswered. What are the implications of recent work on virus-tumours for cancer research generally and are we to expect that human tumours will have a virus implicated in their causation? The second question is not unrelated to that: By what mechanism do viruses cause cancers?

As to the first question, I have already indicated that I shall be surprised if some cancers in man do not turn out to have a virus cause. A virus cause has been discovered for so many tumours lately that one cannot deny that a virus might turn out to be concerned in all tumours. Unfortunately, with the tumours which have come to light it has mostly been a question of new tumours caused by already known viruses rather than of a new virus cause being found for familiar tumours. I find myself right on the fence as to the generality of the implications of the tumour work: I am quite prepared for it to come out either way.

There have been a number of suggestions as to how viruses could cause cancers. They may lead to deletion of an inhibitor which stops the cell from multiplying unrestrainedly. They may become integrated with the genetic material of the nucleus, with the result of what amounts to a somatic mutation; or they may achieve that in some other way. Or they may act upon some hereditary mechanism in the cytoplasm (cf. Dulbecco, 1961). Or as a result of their action there may be a poisoning of certain respiratory enzymes, as suggested by Allison and Lightbown (1961): potential cancer cells might survive the poisoning process where normal cells could not. This last suggestion would mean that there were close analogies between the effects of virus infection and those of known chemical and physical carcinogens. Other hypotheses tend to regard chemical and physical agents rather as unmasking a latent virus—a familiar occurrence in other parts of the virus field.

Does all this get us anywhere with regard to hope of preventing or curing cancer? I feel more hopeful than I used to be. There is, as we have seen, an immunological approach. Yet prevention of cancer along such lines may well be a much more formidable task than preventing common colds, where there are perhaps a mere 30 to 40 viruses to cope with. On the other hand, chemotherapy of virus infections looks much more promising than it did a little while ago. If viruses are concerned in human cancer the hopes are by no means negligible.

I read in the papers of a French plan for a huge International Cancer Research Institute. That will be good only if the attack is broadly conceived. A frontal attack with enormous forces may well fail. The man to make an

important, even crucial, advance may well have been studying chicken-pox and not cancer at all.

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Pyogenic Meningitis in Infancy and Childhood

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This is a study of all the cases of pyogenic meningitis which have occurred in newborn babies, infants, and children up to the age of 12 years in the Sunderland hospital area during the 13-year period 1950–62 inclusive. In this time 337 cases were admitted from a total population of about 400,000. The majority of the cases were treated in Sunderland Children's Hospital and a few at Sunderland Infectious Diseases Hospital. We believe that few, if any, were admitted to hospitals outside this area.

We made this investigation in order to assess the results of treatment over the years, to note if there has been any change in the incidence of bacterial types of meningitis, and to work out a basic plan of management for the future.

Cases have been classified into six groups, depending on bacteriological and clinical findings, and one neonatal group, as follows:

Group 1. Bacteriologically proved meningococcal meningitis.

Group 2. Purulent meningitis. No organism isolated. Petechial rash present.

Group 3. Purulent meningitis. No organisms isolated. No petechial rash.

Group 4. Bacteriologically proved *Haemophilus influenzae* meningitis.

Group 5. Bacteriologically proved pneumococcal meningitis.

Group 6. Miscellaneous purulent meningitis (1 staphylococcal, 1 streptococcal, 1 salmonella).

Group 7. Neonatal meningitis, with symptoms appearing within 28 days of birth.

Detailed Analysis of the 337 Cases

The number of cases in each group and the mortality are shown in Table I. There was no significant sex difference in any group.

TABLE I.—*Total Numbers and Mortality by Groups*

Group	Total No.	Deaths	% Mortality
1	125	2	1·6
2	50	4	8·0
3	100	5	5·0
4	21	4	19·0
5	21	6	28·6
6	3	1	33·3
7	17	7	41·2
Total	337	29	8·6
Excluding neonates ..	320	22	6·9

Table II shows that roughly three out of four cases in any group were under the age of 2 years.

TABLE II.—*Age at Onset of Illness*

Age	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Total
1 month to < 2 years	100	40	74	17	19	2	252
2 years and over ..	25	10	26	4	2	1	68

In the tables that follow we have omitted group 6 because of the small number of cases it contains, and group 7, neonatal meningitis, is analysed separately.

There seems to be a significant increase in the number of meningococcal infections during the winter months (Table III), when respiratory infections are at their height (Heycock and Noble, 1962). This trend was absent in the other groups.

TABLE III.—*Seasonal Incidence. Number of Cases in Each Month*

Group	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1	20	16	11	17	9	10	4	4	6	8	9	11
2	6	4	3	3	5	8	5	3	2	3	5	3
3	11	10	8	6	12	4	12	6	5	3	9	14
4	3	4	0	2	1	2	2	0	1	2	2	2
5	4	2	0	2	3	2	1	0	3	0	4	0
Totals	44	36	22	30	30	26	24	13	17	16	29	30

We sought the yearly incidence of different types of bacterial infection (Table IV) because it has been suggested that there

TABLE IV.—*Incidence of Different Types of Bacterial Infection by Years*

Year	Group 1	Group 2	Group 3	Group 4	Group 5
1950 ..	1	0	4	1	1
1951 ..	3	1	8	1	3
1952 ..	13	2	7	0	2
1953 ..	13	7	8	2	6
1954 ..	13	3	12	0	1
1955 ..	17	5	6	0	0
1956 ..	7	1	7	3	1
1957 ..	11	3	6	3	1
1958 ..	13	6	10	2	1
1959 ..	8	7	10	0	2
1960 ..	9	2	8	3	0
1961 ..	10	3	9	3	2
1962 ..	7	10	5	3	1

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