Section of Comparative Medicine

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[*November* 1, 1939]

Latent Virus Infections and Their Possible Relevance to the Cancer Problem

PRESIDENT'S ADDRESS

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ANIMALS and plants may harbour viruses within their cells, though showing no evidence of disease; bacteria, too, may carry a bacteriophage which does not obviously affect them. Such phenomena may be called "latent virus infections".

VARIETIES OF LATENT VIRUS INFECTIONS

Virus infections which do not obviously affect the host may not all have the same natural history. Let us consider the several varieties; we shall note that they are not sharply separable from one another.

(i) *Transient Latent Infections*

Under this heading we include those diseases in which an animal becomes infected with a parasite, the infection runs its course and the parasite ordinarily ceases after a time to be recognizable; at no stage, however, do overt symptoms of disease occur. We shall see later that a small quantity of residual virus may be very hard to demonstrate in a recovered animal, and non-recognition of a virus may thus be due to the imperfections of our technique. It would be more accurate, therefore, to call our first group "Apparently transient latent infections".

The natural subclinical immunization of many human beings against diphtheria may be ascribed to transient latent infection, and there are many examples to be found amongst virus diseases. For example, yellow fever occurs in a wide area of tropical America and Africa and convalescents from the disease have in their sera neutralizing antibodies active against the virus. But many of the inhabitants of these regions have antibodies in their sera though they have never suffered from classical yellow fever. Some may have undergone an attack of yellow fever manifesting itself as an influenza-like infection and of these 90% have yellow fever antibodies, but of those who do not even give this history, 47% have specifically neutralizing sera (Soper and de Andrade, 1933). There is no dispute that yellow fever antibodies occur only as a specific reaction to infection with the virus, and it thus appears that yellow fever can exist as a symptomless infection.

During the course of influenza epidemics, sera of many persons in the epidemic areas show substantial rises in neutralizing antibodies against the virus though the people concerned have remained in perfect health throughout the epidemic (Stuart-Harris, Andrewes and Smith, 1938; Francis, *et al.*, 1937). The majority of human adults, too—80% in American cities—have antibodies against poliomyelitis virus in their blood: their significance is disputed more than that of the antibodies active against yellow fever and influenza, but most workers agree that they probably indicate past contact with, and often inapparent infection with, the virus of infantile paralysis. Amongst diseases of birds we may notice psittacosis. According to Meyer and Eddie (1934), a normal course of events is for budgerigar nestlings in aviaries in California to develop a symptomless infection with psittacosis virus; this is

associated with enlargement of the spleen from which virus is usually not recoverable after the sixth or eighth month of life.

In the examples just quoted the infecting agent is capable of producing, according to circumstances, a clinical or a subclinical infection in the species attacked. Nicolle and Lebailly (1919) used the term " infection inapparente " to denote particularly an infection which in a particular species was always symptomless. Thus, the Rickettsiæ of typhus fever which they studied produced no symptoms in the rat, could be carried on indefinitely by rat-to-rat passage without the production of symptoms but would cause symptoms again on inoculation back to the guinea-pig. Burnet (1936) has described the reaction of the rat to louping-ill virus. In this instance, virus introduced up the nose of a rat will pass to the olfactory bulbs but as a rule no further ; no symptoms are produced and continued passage in series is not possible. This would seem to be a true instance of " infection inapparente " in Nicolle's sense, but Burnet and most English writers use the term inapparent infection as synonymous with latent infection. The distinction is not clearly defined and it seems better, therefore, not to treat " infections inapparentes " as a separate category of latent infection.

(ii) *Persisting Virus Infections*

After an overt attack of a virus disease, the responsible virus may continue to be harboured by a host at a time when the disease-symptoms have long disappeared. Poliomyelitis virus has been recovered from nasal washings of man during convalescence, and the very similar virus of mouse-paralysis (Theiler, 1934) has been found in the spinal cords of recovered mice for as long as a year after infection. Fowls infected with the virus of fowl-pox continue to carry the virus for long periods, particularly in the liver, and after infection with laryngo-tracheitis (Gibbs, 1933), the virus of that disease may be found in the trachea of fowls for as long as two years. Since most animals infected with a virus develop antibodies against that virus in the course of their disease, it may be a hard task to demonstrate a small quantity of persisting virus in the tissues of a recovered animal, since it may be neutralized by the antibodies present when the tissues to be tested are ground up and extracted. Special techniques have been devised for overcoming this difficulty ; Olitsky separated virus from the antibody in recovered animals by cataphoresis and thus revealed the presence of vaccinia virus in the tissues of convalescent rabbits for as long as 133 days after infection. The virus of foot and mouth disease was detected in the urine of hyperimmunized cattle 246 days after infection (Waldmann, Trautwein, and Pyl, 1931) by a method involving adsorption of virus on to charcoal ; here, however, they were probably dealing with concentration of virus and not solely with separation from antibody.

In some instances potent antibodies are not recognizable in the recovered animal and then virus may be revealed more easily. Traub (1938) could as a rule find no antibodies in the sera of mice recovered from lymphocytic choriomeningitis infection and he had no difficulty in showing that virus was present for long periods in many such animals. In the case of psittacosis-infected mice, Bedson (1938) was able to find virus in the spleen for seven months ; psittacosis is another disease in which potent neutralizing antibodies are hard to demonstrate. A classical example of persistent virus infection is equine infectious anæmia. A horse may he attacked by this virus and may show comparatively trifling symptoms or none at all over a period of years ; and yet a few drops of its blood taken as long as fourteen years after the original infection and injected into another horse have been known to produce a fatal infection ; here, too, antibody production is not in evidence (Schalk and Roderik, 1923).

Many writers on viruses have sought to explain the long-continued immunity which follows many virus infections as being an infection-immunity, an immunity which only persists because the virus has never completely died out of the body. Of recent workers Webster (1938) suggests that the immunity of mice

vaccinated against St. Louis encephalitis virus may only endure while virus is still present, particularly in the spleen, while Bedson (1938) has made similar suggestions concerning the immunity of mice to psittacosis. Where virus can be shown to persist for a long time it is easy to believe that such persistence is responsible for the long immunity. Even where no virus can be recognized, its presence might very well be suspected, though masked by co-existing antibody. It is very possible that persistence of virus is the rule and yet we can only recognize that persistence is only possible when neutralizing antibodies cannot act to hide the virus. We must, however, be cautious and not claim that persisting virus is always the cause of long-enduring immunity until we are surer of our ground.

(iii) *"Indigenous" Viruses*

Interesting as are transient latent infections, which are apparently self-limited, and the instances of viruses persisting after overt infections, a third group is perhaps more significant still. These may be called "indigenous viruses". The group includes those viruses which in their behaviour combine the properties of the first two groups: as in our first group they cause symptomless infections, which, however, are not self-limited; as in the second group the viruses persist indefinitely in the host. There is a third characteristic of "indigenous viruses": they commonly infect their host very early in its life. They may make their entry in infancy or childhood, or *in utero* or may even be handed down from the parent in the germ-plasm. Thus an animal or plant may be born with a virus-infection of which the presence can only be recognized indirectly. One may by introducing some stimulus, upset the virus-host equilibrium, and cause the appearance of obvious disease or one may transfer the infection to a susceptible individual, an "indicator host". (This term will be discussed later.) This type of host-parasite relationship is what Theobald Smith (1934) has considered as perfect parasitism, an association in which the two partners are perfectly adapted to one another, neither causing the other any inconvenience and with admirable arrangements for perpetuating the partnership from one generation to the next. Of this nature are the symbionts which are found in the cells of many insects: they are believed on morphological grounds to be usually bacterial in nature or perhaps related to Rickettsiæ, and some at least have been cultivated on artificial media (Glaser, 1930). They may be transmitted "through the egg", but the transmission is rather "on the egg" than actually through the germ-plasm; eggs are contaminated by the symbiotic agents which are present in the ovary at some time before the eggs are laid.

Lysogenic Bacteria

There are now overwhelming grounds for believing that the bacteriophages are of the same nature as viruses, are in fact viruses which parasitize bacteria. It is also established that many bacteria, so-called lysogenic bacteria, regularly yield filtrates containing a phage, active, not upon the strain "carrying" them, but upon some other sensitive strain. Such a strain can be used to test for the presence of phage and is therefore called an indicator strain. Many strains of *B. coli* for instance carry phages which are active on Shiga dysentery bacilli (Lisbonne and Carrère, 1922); *B. sanguinarium*, again, is a very valuable indicator organism for lysogenic Salmonellas (Burnet, 1932). For some time it was supposed that diphtheria-bacilli were not subject to phage-action; then Smith and Jordan (1931) showed that all diphtheria bacilli were lysogenic, carrying phages active against an indicator strain of *C. diphtheriæ*. The diphtheria phages had not been detected before simply because no indicator strain had been discovered.

Burnet (1932) showed that nearly all Salmonellas were lysogenic, carrying one or more of several different phages. He has pointed out that the multiplication of phage and bacterium must be exactly co-ordinated so that each daughter-cell receives some phage when cell division occurs. Lysogenic bacteria afford an excellent example of the indigenous virus, an example, moreover, in which the virus is handed down in the germ-plasm—in so far as we can use the term germ-plasm with respect to a

unicellular organism. Why it is that a lysogenic bacterium is not lysed by the phage it carries we do not know. Does it lack some structural element which the phage must attack before it can dissolve the bacterial wall ? Is there some little understood mechanism for intracellular restraint of the parasite ? And if so does this act by preventing the phage from building up its concentration to the threshold for lysis ? An answer to these questions might prove of the utmost importance in the virus field generally.

It seems clear in any case that the phage-bacterium equilibrium is not necessarily wholly stable. Cultures of certain lysogenic bacteria may exhibit " nibbling " of the colony edges from time to time, a phenomenon suggesting that the bacteria, or at any rate some variants occurring in the culture, are not wholly insusceptible to the phage which is being carried. Curious appearances which turn up in cultures of *B. pyocyaneus* can also be best explained in terms of incomplete resistance on the part of lysogenic organisms. Lominski (1938) has described bacterium-phage associations of varying sorts : one may find frank lysis of a culture, and lysogenic strains. But besides these are attenuated lysogenic strains, strains in which the only demonstrable effect of the phage which is carried is to render the bacterium resistant to the action of a related but more virulent phage. Or again in a " crypto-lysogenic " strain the lysogenic character of the culture may be wholly hidden unless one alters the environment by making the culture grow under difficulties, when evidence that a phage is being carried may reappear.

We shall discuss later how such upsets of a host-parasite equilibrium may be important in explaining phenomena of disease in animals.

Indigenous Plant-viruses

It has been shown by Johnson (1925) that almost all strains of potato under cultivation in America " carry " a virus, for extracts of American potato plants inoculated into tobacco and certain other solanaceous plants will regularly produce a disease having the properties of a virus infection. In fact it is possible that " normal " potatoes may carry more than one virus. Such virus commonly produces no symptoms in potatoes ; but after several passages through tobacco its virulence may become exalted until it can cause disease when inoculated back to its original host. In calling this an indigenous virus we must bear in mind that vegetative reproduction is the rule in the artificial propagation of potatoes and that potatoes raised from true seed do not apparently carry the virus from their youngest stages. Latent plant virus infections exhibit one important feature : they may render the host-plant refractory to infection with a related but more virulent strain of virus (Salaman, 1933).

There is some evidence that with higher plants, as with bacteria, change in environment may upset the virus-host balance ; thus the viruses of crinkle and potato mosaic produce symptomless infections when grown at over 20° C. but characteristic symptoms when the temperature is reduced below that level. In other instances abnormal cooling masks symptoms, as when tobacco plants infected with mosaic are grown at temperatures below 7° C. (Bawden, 1939).

Indigenous Viruses of Animals

Most animals differ from plants and bacteria in their ability to form antibodies, and we have already seen how viruses persisting after an attack of disease may remain masked by co-existing antibody. It might therefore be expected that the presence of indigenous viruses would be harder to detect in the animal than in the vegetable kingdom. One or two interesting examples are, however, known to us.

(*a*) *Lymphocytic choriomeningitis in mice.*—It is doubtful whether the virus of this disease is a mouse virus which occasionally infects man or a human virus which has managed to establish itself in mice. The equilibrium established between this parasite and a colony of mice has been described in some very important work by Traub (1939). In his earlier studies he noted that virus persisted in the blood of

mice after recovery from infection and that female mice thus carrying virus could infect their young *in utero*. Such young mice showed symptoms of disease, particularly tremors and inco-ordination. Other young mice not infected *in utero* became infected by contact soon after birth and showed no definite symptoms, only a decreased growth-rate. In such mice it was possible at a certain stage of the infection to produce frank symptoms by the intracerebral inoculation of sterile broth—another result of disturbing a host-virus equilibrium. Dr. G. M. Findlay tells me that one stock of mice in England carries choriomeningitis virus and it is possible to provoke symptoms in them with some regularity by intracerebral broth-injection. Let us return to Traub's mice: as virus and mice lived together for two years the state of affairs gradually changed. Infection of young mice *in utero* became the invariable rule, apparently because all stock mice, young and old, were now carriers. Further, the disease had gradually become milder till it produced no symptoms at all; all mice carried virus from birth and had an infection-immunity throughout life. Virus could only be demonstrated in them by inoculating tissue extracts into another strain of mice which carried no virus and were not immune; these afford a rare instance in animal pathology in which the investigator has available an "indicator host". So far as Traub could determine, the changes in the epidemiology of the disease were due to a decreased pathogenicity of the virus for mouse embryonic tissue, permitting more and more mice infected *in utero* to survive into adult life. He had noted that the earlier in life infection occurred, the greater was the virus content of the nasal secretions, and the greater the infectivity of the mice for contacts and the longer the carrier-state. Conditions thus gradually became more and more perfect for ensuring that all mice became carriers and hence that all young mice were infected before birth.

Choriomeningitis affords an extraordinary example of an indigenous virus infection of an animal; for it has been possible to trace to a large extent the evolution of the state of "perfect parasitism" taking place during the course of a few years. The facts have probably been easier to trace because of the circumstance, already mentioned, that mice do not develop readily demonstrable neutralizing antibodies against the virus in question.

(*b*) *Herpes simplex.*—Fever blisters in man may notoriously be elicited by a number of stimuli, in some persons by "colds" and various fevers, in others by ultra-violet light, menstruation, or eating cheese. It has been the general view that these stimuli act by lighting up a latent infection with the virus of herpes, but the epidemiology of the disease has been lately put into sharper focus by Burnet and Lush (1939). Dodd, Johnston and Buddingh (1938), found that herpes virus was responsible for many cases of infectious stomatitis in young children and Burnet has confirmed this. He also confirmed some observations by Andrewes and Carmichael (1930) and by Brain (1932) indicating that those people who were liable to recurrent attacks of herpes carried potent antibodies in their sera, while adults not so liable had no antibodies. There was a sharp division into two categories, those who had large amounts of antibody and those who had none; people from the less favoured social strata tended to be in the former group. Burnet interprets this and other contributory evidence as follows:—many children, particularly those of poorer families, suffer from herpetic stomatitis before they are 5 years old. In the course of this infection they develop in their sera neutralizing antibodies which were not present before, and the titre of these antibodies then remains fairly stable throughout life. This persistence of antibody is probably associated with persistence of virus; where in the body this lies hidden is uncertain, though Levaditi, Harvier and Nicolau (1922) found virus in the mouth washings of "normal" persons. Burnet suggests that herpes virus lies latent somewhere in the central nervous system, though he himself failed to find it in what he thought a likely spot, the Gasserian ganglion. At any rate it seems that the habitual "herpetiker", as Doerr calls him, carries virus somewhere about him, and that the antibodies associated with his carrier state are

unavailing to protect him from a local herpes eruption when a suitable provoking stimulus occurs. The effective stimulus is different in different subjects and may have to be a strong one such as does not occur often, for some people who have antibodies are only very rarely afflicted by fever blisters.

Herpetic stomatitis has not been recognized, at least not commonly, in adults; the percentage of herpes-carriers or persons with antibodies does not seem to rise after early childhood is passed: apparently human beings become less susceptible to natural infection with herpes after the age of 5, though experimentally most persons, even adults, *can* be infected by intradermal inoculation of large doses of virus. The greater susceptibility of the very young animal to certain viruses may be important in our attempt to unravel this "indigenous virus" question. It may often be obscured by the protective action of maternal antibodies in the animal's early life, and there may in other instances be an increased resistance of young animals apart from such antibodies. It has already been seen, however, that mice infected with choriomeningitis virus *in utero* are more severely affected than those infected after birth. Young mice also are particularly susceptible to influenza virus and the virus of St. Louis encephalitis. Guinea-pigs and rabbits, which are relatively resistant to influenza virus, may be readily infected *in utero* (Woolpert, 1939). Very young chicks infected with Rous sarcoma virus may be laid low by a rapidly fatal generalized disease without neoplastic characters (Duran-Reynals, 1939). Another example of the same phenomenon is the susceptibility of chick embryos to a number of viruses to which the newly hatched chick is resistant. It need not follow that a sudden decline in susceptibility occurs at birth or hatching: in the case of herpes, liability to infection apparently wanes during the first few years of life. It may be noted that young plants and actively growing bacteria also tend to be more susceptible to infection with viruses (or phages) than older ones in less active growth.

(*c*) *Virus III of rabbits and mouse-pneumonia viruses.*—Rivers and Tillett (1923) found that after a few serial intratesticular passages of human varicella material through rabbits one obtained an acute orchitis, the agent responsible for which was clearly a virus, now known as Virus III. They were soon led to doubt the relation of this virus to varicella; and Andrewes and Miller (1924) recovered the same virus by similar serial passages through rabbit testes, starting with normal human blood. Rivers and Tillett (1924) found that 10–15% of American rabbits were resistant to the virus and about the same percentage had neutralizing antibodies in their sera. They concluded that Virus III was an indigenous virus of rabbits and that it was brought to light by the technique they used. In 1928 I could find no evidence that the virus was present in domestic rabbits in London (Andrewes, 1928), though it has turned up here since, in 1938.

A similar story has to be told now that passage of viruses by intranasal inoculation of mice has become a popular technique. Dochez *et al.* (1937), Gordon, Freeman and Clampit (1938), and Horsfall and Hahn (1939), have thus brought to light viruses causing pneumonia in mice. The agents described by these authors are not necessarily the same, but in each instance some material or other has been dropped up the noses of mice, their lungs have been later harvested and emulsions thereof dropped up the noses of other mice. After a few passages, pneumonia has appeared and it has been easy to show that this is caused by a virus transmissible in series. Horsfall and Hahn have been able to recover their virus from certain strains of white (Swiss) mice but not from others; all of the Swiss mice originally came from the same source. The strains of mice from which the virus is not recoverable are much less sensitive to it. One may wonder whether their freedom from it is due to an inherent resistance—yet they are presumably very similar genetically; or whether the resistant mice may not have an infection-immunity such as Traub's choriomeningitis mice all had when, in 1937, a stable virus-host equilibrium seemed to be established.

One naturally wonders how this technique of serial passage through apparently normal animals can act to bring to light a wholly hidden virus. Most probably the technique simply permits one to obtain virus in an abnormally high concentration. Some viruses—certain strains of influenza for example—produce a symptomless infection when given in very small doses and typical symptoms when inoculated in larger quantity. The mouse-pneumonia viruses and Virus III may normally produce a subclinical infection and never build up to a high titre. But rapid passage under favourable conditions could conceivably, by keeping them artificially, as it were, in a permanent logarithmic phase of growth, allow them to attain a far higher concentration than is normally possible, a concentration such that they could produce obvious pathological changes and symptoms of disease. Possibly the change is not merely quantitative: the unusually favourable environment may permit a mutation of the virus, though of this we have no evidence. Study of this laboratory phenomenon of the production of a disease from an inapparent infection by rapid passages must make the epidemiologist think of analogies in his field. Here it often seems that provision of opportunities for rapid passage of an agent from one individual to another in nature may create an epidemic out of nothing.

(iv) *Complicated Latent Infections—Swine Influenza*

Let us now consider a latent infection of, perhaps, a special kind. Shope (1939) has recently brought forward evidence which may bear on our theme; he has suggested that the virus of swine influenza may pass through an intermediate host in lungworms. The embryonated ova of lungworms from a pig are passed in the pig's fæces, and some of them are later ingested by earthworms in which the lungworms in question pass some stages in their life-cycle. At a later date the earthworms are eaten by other pigs and the lungworms find their way through the intestinal walls back to the pig's lungs. Now the lungworms in a pig with swine influenza may take up or be contaminated with the swine influenza virus, and their embryonated ova, hatching out after being taken up by earthworms, may carry virus with them through their life-cycle back to another pig when he, in due course, eats the earthworms. The virus-carrying lungworms will thus reach the susceptible tissues of a pig's lungs. But this event is not enough to make a pig go down forthwith with swine influenza. The virus will lie harmlessly in the worms until some provoking stimulus lights the infection up in some quite obscure fashion, and the pig *does* get influenza. An injection into the pig's muscles of a culture of *Hæmophilus influenzæ suis* has been the provoking stimulus chiefly used. These are Shope's interpretations of his findings; his results need further study and confirmation before rash conclusions are drawn. It appears possible, however, that in this instance we have a virus lying latent not in the host's own tissues, but in those of an animal parasite, in which they are revealed after a provoking stimulus. This provocation does not act, however, merely by mechanical liberation of virus from the lungworm, for Shope has not yet demonstrated virus by injection into pigs of ground-up "infected" lungworms; it would seem possible that virus is present in the worms in some altered state. Shope's stimulating suggestions are noted at this point in order to point out that latency of a virus may be a complicated affair.

LATENT VIRUSES AND CANCER

Theories about cancer which claim a rôle for virus in the ætiology must postulate that such a virus, or viruses, are very widely distributed in the animal kingdom, that they are normally latent infections, but are lit up by some stimulus such as the application of a carcinogenic hydrocarbon. Those who decline seriously to consider such a view do so partly on the ground that a ubiquitous latent infection with a virus is "absurd". Enough has been said in the first part of this address to show that ubiquitous latent virus-infections come into the realm of known facts; and facts are absurd only to those who do not understand them. There is admittedly very little direct concrete evidence that cancer in general is

caused in the manner suggested. But I can draw your attention to some facts in experimental cancer research which seem to me strongly reminiscent of some things we have just discussed in the field of virus-diseases.

(*a*) *Fowl paralysis* (neurolymphomatosis).—Let us first glance at fowl paralysis, an obscure disease which is certainly related to neoplasms. As you are aware, the paralysis in this disease is associated with great enlargement of the nerves, due to massive infiltration with round cells. Lymphomatous tumours seem often to arise from the collections of round cells associated with the typical lesions; and a strain has been described by Furth (1934) which on propagation yielded sometimes lymphomatous nerve lesions, sometimes myelomatosis and sometimes endothelioma. Dalling stated recently that there were three theories of the nature of fowl paralysis: that it was an infection, a tumour or a virus-disease. While reluctant to introduce theology and particularly the Athanasian creed into pathology, I would suggest that we have here not three explanations but one explanation, not three theories but one theory; an "infection with a tumour virus" would serve as an explanation which could cover all the facts. Evidence that an infective agent was concerned has hitherto been inconclusive, but Blakemore's (1939) recent studies have marked a definite advance. From a stock of fowls subject to fowl paralysis he obtained by in-breeding a strain free from the disease yet highly susceptible to inoculation with infectious material from diseased birds—in fact a perfectly good indicator-strain. Inoculations of fowl paralysis material into his indicator hosts produced either nothing very much or merely "unthriftiness" associated with lesions in the heart and liver and occasionally true fowl paralysis. But further passage raised the virulence of the infective agent and gave rise to an acute disease, the lesions of which were at first inflammatory, later often lymphomatous. Evidence was obtained suggesting that fowl paralysis is the chronic stage of an acute disease which may naturally be wholly or largely symptomless. There is yet no proof that the agent is a virus, but analogy with similar conditions in fowls, such as leukæmia and sarcoma, makes it very probable. It seems fairly certain that the agent can be transmitted from the mother through the egg; there are also reasons for believing that purchased cockerels carrying a latent infection may have passed the disease on to their progeny (Blakemore, 1934–35). There is also evidence that environmental factors may determine whether or not a latent infection will blossom out into declared disease: chicks from infected stock have been divided into two lots and those taken and reared at one farm have developed symptoms while those kept at another have not.

(*b*) *Tar-sarcomata in fowls.*—Most normal fowls develop as they grow older neutralizing antibodies active against filtrates of Rous sarcoma virus; the antibodies are, however, usually low in titre compared with those which one finds in birds bearing slow-growing tumours. Their presence suggests, though it does not prove, that many normal fowls may carry or have had contact with a virus serologically related to fowl tumour viruses such as that described by Rous. McIntosh (1933) reported that a number of fowls injected intramuscularly with tar developed sarcomata and that in three instances the resulting tumours could be propagated with filtrates. The filtrable agents had the properties of a virus. Recently McIntosh and Selbie (1939) have obtained two more filtrable tumours in tarred fowls arising at the site of inoculation of the tar. No similar tumours appeared in birds of the same age not treated with tar. The suggestion naturally arises that the tar injected into these fowls has activated an indigenous virus of the Rous-virus family. Other workers, however, have produced tar-sarcomata in fowls and have failed to obtain active filtrates. A possible clue to these diverging results is afforded by some work of my own (Andrewes, 1936) and of Foulds (1937). A tar sarcoma was produced in a fowl by Mellanby and this, though transplantable with cell-grafts, has always resisted efforts at transmission with filtrates. The tumour would, however, grow on inoculation into pheasants; sometimes very large tumours

formed. The inoculated pheasants regularly developed neutralizing antibodies active against filtrates of Rous No. 1 sarcoma ; injections of normal chick embryos caused no such antibodies to form. Fowls grafted with this tar sarcoma also developed potent antibodies to the Rous virus (Andrewes, 1939). The suggestion is a strong one that the non-filtrable sarcoma contains a virus serologically related to Rous virus though not directly demonstrable by filtration experiments. It must be mentioned that the filtrable agents of various histologically distinct fowl tumours have been shown to be related serologically (Andrewes, 1931) so that cross-neutralization of the kind suggested would not be surprising. Foulds (1937) obtained similar findings with a non-filtrable dibenzanthracene sarcoma in a fowl ; he was able to elicit anti-Rous properties in the sera of rabbits by injections of crude or even filtered extracts of this non-filtrable tumour. It may be, therefore, that tumours induced in fowls by injections of tar or other carcinogens may generally owe their continuing malignant character to the action of a tumour-virus within the cells, such a virus having been liberated from some restraint by the poisoning of the cells by the tar. It may be that this liberation from restraint is only sometimes sufficient, as in McIntosh's experiments, to allow the virus to infect normal cells : possibly the virus which we imagine to be carried by McIntosh's strain of fowls is more easily exalted in virulence than the indigenous fowl tumour viruses present in the fowls studied by other workers.

To suggest that a carcinogenic agent can upset a cell-virus equilibrium in the manner postulated is not to theorize entirely beyond the known facts. For as Ahlström and I showed (1938) tar and other carcinogens can apparently act in just such a manner in rabbits infected with Shope's infectious fibroma virus. Normally, this virus causes a proliferation of fibroblasts leading to the formation of sarcoma-like tumours ; but these always regress after a few weeks. In rabbits treated with tar the regression is delayed, often for months, the "tumours" become locally invasive and rabbits may even die with generalized fibromatous lesions. Carcinogens do not have this dramatic effect on the course of vaccinia and other virus diseases which are not associated with great proliferation of cells. It may be recalled in this same connexion that another rabbit-virus, the papilloma virus also described by Shope, produces warts on rabbit's ears and that these may become malignant, but only after many months. After previous preparation of the ears with tar, however, the same virus produces growths many of which may be malignant almost from their first appearance (Rous and Kidd, 1938). How the tar acts we do not yet understand : it may do so through an upset of a cell-virus equilibrium such as would seem to explain the phenomena in McIntosh's fowls and in our tarred fibroma rabbits.

Bittner's Experiments with Breast-carcinoma in Mice

Some strains of mice used in cancer-research have an incidence of breast cancer in breeding females of 80–90%, while in others the incidence is almost nil. In hybrids between two such strains it has been found that the incidence of breast cancer in the offspring depends not upon Mendelian rules but wholly upon whether the mother came from a high or low cancer family : only the mice born from high cancer mothers developed breast cancer in their later life. Bittner (1939) found that mice from the low cancer strain would develop a high proportion of cancer if they were suckled from birth by high cancer-strain mothers. Conversely, mice from high cancer mothers would have quite a low tumour incidence if they were fostered by mothers of the low cancer strain. It appeared as if something affecting the occurrence of cancer in later life was transferred to the young in the mother's milk. But still odder findings were to come : if young mice of the low cancer stock were suckled by high cancer mothers and consequently developed cancer later despite their hitherto unstained family escutcheon, then, on inbreeding them their children and grandchildren were also very liable to breast-cancer : the blot on the escutcheon was passed on. On the other hand,

young of the high cancer stock which escaped the disease through being fostered by low cancer mothers were able to pass on their freedom from cancer to their descendants. Bittner has considered whether this agent transmitted in the milk may not be a hormone; I can swallow some things, but it is hard to swallow a hormone which, when taken in infancy will condemn your grandchildren to the development of cancer in their years of maturity. On the other hand, if we try to explain the phenomena in terms of viruses, if we cast our minds back to some of the instances of indigenous virus infections, we find that choriomeningitis and mouse pneumonia viruses are present almost universally in certain strains of mice but not in others; that choriomeningitis and herpes viruses can apparently infect the very young animal more readily than the adolescent or adult, though it is true that no example of transmission of the agent in the mother's milk was described; that, again in choriomeningitis, the infection may be made manifest only by the giving of a non-specific stimulus in later life. Such references to known phenomena in the virus field make it not too difficult to believe that Bittner's high cancer mice are infected with an indigenous virus, which normally remains quite latent. But ultimately a series of stimuli, perhaps acting over a long period, reveal its rôle as a causative agent of breast carcinoma. It might be expected that an agent with a habitat in the breast might be transmitted in the milk and that mice not infected in their young, highly susceptible state might escape infection when they had grown refractory with increasing age. There is, we may note in passing, evidence that infection through the milk does not occur with other mouse-tumours, notably lung-carcinomata.

"*Toothless Viruses*"

We have in our discussion come across examples of viruses which cannot be demonstrated directly by injection into fresh hosts, but only by roundabout means. The evidence for presence of a virus in non-filtrable sarcomata in fowls was based on serological evidence. Purely immunological, also, is the evidence that a virus is present in most rabbit papillomata in domestic rabbits and in the cancers developing therefrom. While papillomata in wild cottontail rabbits usually yield extracts which will readily infect either wild or tame rabbits, the warts which appear on domestic rabbits do not as a rule do so. Even in extensive, progressively growing, papillomata in domestic rabbits one usually cannot reveal directly by transmission experiments that virus is present: but extracts of the warts will immunize other rabbits effectively when injected intraperitoneally, leaving little doubt that plenty of virus is present in some masked form (Shope, 1937). Again, the carcinomata derived from such warts may be transplanted to other rabbits; when the grafts grow they lead to the development of neutralizing antibodies to papilloma virus in the rabbits' sera—further evidence that an occult virus is present (Kidd, Beard and Rous, 1936). Shope's swine-influenza experiments, also, suggest that virus in the lung-worms is not necessarily present in the normal, fully infective state.

Such findings make one wonder whether a virus may not depend for its power to infect normal cells on some, possibly haptene-like, 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, as armadillos and ant-eaters have done. This could happen most readily, one may imagine, in the environment of the cancer-cell, where virus could be carried on 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-extracts into fresh hosts. Roundabout methods of revealing them would always be necessary. In the instance of Bittner's mouse-carcinomata the toothlessness would presumably not be absolute; a single incisor would perhaps remain, adequate only to permit entry to the peculiarly susceptible cells of the tender infant mice. 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. Complete toothlessness would seem to involve a transmission through the germ-plasm : relative toothlessness would allow a little more latitude. Toothless viruses need not be found only in cancers. There are the viruses which, as some workers think, *must* persist after infections and be responsible for keeping up a life-long immunity and yet which obstinately refuse to be demonstrable ; may they too not be modified, relatively edentulous, instead of being merely masked by antibody ?

Green (1938) has suggested that a cancer-virus in a cancer-cell is the highest conceivable form of parasitism, virus and cell having their division exactly synchronized, almost a virus-host hybridization. I would disagree. The perfect parasitism is rather the association of a latent indigenous virus and its host, neither doing any harm to the other. Such a compromise has probably evolved in more instances than we suspect during the struggles between viruses and their hosts. Cancer, when it occurs in animals, would then be due to the incursion of some unexpected factor, a *tertium quid*, which broke up the happy association ; a disease affecting both partners, of advantage to neither. Instead of a disease of man caused by a virus, we should then have to consider human cancer a disease of the man-virus partnership.

I have not attempted to-day to report anything wholly new. I have tried rather to bring together some facts which have impressed my roving eye as it has tried to keep up with a small part of current literature. There are facts about bacteriophages, about plant-viruses, about neurotropic animal viruses, about cancer, which do seem to hang together, to fit into the same sort of general pattern. We have noted that the association of hosts and parasites, particularly viruses, may lead not to violent disease but to a certain balance of power. We have observed that such a balance may at times be upset, with the result that disease occurs after all. Finally we have been led to wonder whether such an upset may not be the fundamental cause of a particular disease, cancer.

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Discussion.—Dr. THOMAS LUMSDEN wished to refer to only one statement. Dr. Andrewes had said that Bittner's experiments provided an instance of the transmission of a latent virus since, when young mice of a low cancer strain were nursed by a high cancer-strain mother, the young mice became prone to cancer just as the young of high cancer-strain mice are when normally nursed by their own mother. Dr. Lumsden gathered from talking with Bittner recently that there was no constancy in this side of his experiments. It was generally admitted that the converse was true—namely that young of a high cancer strain, fostered within the first twenty-four to forty-eight hours on low cancer-strain mothers, developed some resistance to the cancer they would naturally have been prone to, but the opposite did not hold and so there was in this case no direct evidence of the transmission of a virus.

Dr. G. M. FINDLAY said that although in one species of animal a virus might produce an active disease, in other species it might be entirely latent. Thus in West and Central Africa about 20-25% of wild monkeys were found to contain immune bodies to yellow fever in their blood. If, however, yellow fever virus was injected into non-immune African monkeys no clinical reaction of any sort occurred. Considerable variation might also occur in the reaction of individuals of the same species to a virus infection. Thus in the majority of persons, Rift Valley fever virus induced a short but unpleasant febrile attack. The speaker, however, had become immune to Rift Valley fever some nine years ago without any sort of clinical attack. An example of a latent virus being excreted in the milk after an acute attack was equine infectious anæmia. Intestinal excretion could also be continued for some time since an instance was known in America where psittacosis virus had been excreted by a parrot for at least eighteen months.

Dr. TOM HARE said that the President had given prominence to certain authors who claimed that fowl paralysis was an infection due solely to a filtrable virus. He, himself, had formed the impression that fowl paralysis, the pathology of which resembled that of neurofibromatosis in man, was inherited as a recessive. Was the President satisfied that in fowl paralysis a virus was transmitted through the germ plasm without explaining how a non-contagious virus was transmitted in mendelian ratios ? He (Dr. Hare) contended that the evidence for the inheritance of fowl paralysis could not be ignored. If it should be shown that inheritance did not play the whole part in causing fowl paralysis, he would suggest that if a virus contributed to the neoplastic process it did so only in those birds which inherited a tissue susceptibility.

The PRESIDENT (in reply to Dr. Lumsden) said that his description of the mammary-cancer work had been based on Bittner's published data. Answering Dr. Hare, he declined to elaborate a theory of the inheritance of viruses as recessives until confronted with evidence that such a mode of transmission did in fact exist.