THE PANTON MEMORIAL LECTURE FOR 1955

THE MODE OF VIRUS MULTIPLICATION AND THE SUSCEPTIBILITY OF THESE AGENTS TO THE ANTIBIOTICS

BY

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It would be true to say, I suppose, that before the last war Panton was hardly known outside the London Hospital. He rarely if ever attended scientific meetings and published little. At the London, however, where from small beginnings he had built a busy department of clinical pathology, he was known as a pathologist of wide experience and sound judgment and a wise administrator. He was my chief when I went there in 1926, and I count myself fortunate to have been associated with so stimulating and delightful a colleague. For a time after the outbreak of war in 1939 Panton was in charge of the laboratory services in the London Hospital sector in Middlesex and Hertfordshire, but the need for someone at the centre to advise on questions of hospital pathology soon led to his being moved to the Ministry of Health. There as consultant adviser in pathology he set himself to plan with energy not only for the immediate needs of the Emergency Medical Service but also for the State hospital service which most of us believed would come with the restoration of peace. And so well did he plan that when the National Health Service was launched in 1948 little more than finishing touches were necessary to complete the hospital pathology service; it stands to-day as a monument to his wisdom and vision. What more fitting memorial to Panton than this one which he himself created? Why institute a lecture to his memory when all that he did for hospital pathology is already so well commemorated? With this I would agree were it not that I discern a tendency to forget how much we owe to this man. It is barely six years since Panton left the Ministry of Health and began a well-earned retirement which was to prove so short-lived. He died on December 27, 1950, worn out by the unremitting labour of the last 10 years of his life, and yet already the memory of all that he did for clinical pathology seems to be growing dim, and amongst the younger pathologists there are some who have not even heard of Panton. It

is because of this that I applaud the action of the Association of Clinical Pathologists in instituting this lecture which it is my privilege to deliver this afternoon. And although I am beset by doubts as to my ability to do justice to the occasion I am grateful to the Association for giving me the opportunity of doing honour to this man whom I held in such esteem and affection.

When I accepted the invitation to give this lecture it was suggested to me that a general topic such as the development of clinical pathology in this country and the part played in this by Panton would be very apposite. It would give me the opportunity for being anecdotal and so creating a picture of this man for those who had not been fortunate enough to know him. But I mistrust my ability to make a success of such a theme, so I have put this idea from me and have selected a more academic subject for my lecture, the question of the bearing of the mode of viral multiplication on the susceptibility of these infective agents to chemotherapy. Even this choice strikes me as somewhat daring. The nature of viral multiplication is a most controversial subject. Already the literature dealing with it is very considerable, and I could not claim either to have read more than a fraction of the papers published on this subject, or perhaps fully to have understood all those that I have read. But it is a problem which interests me very greatly, and its importance both academically and from the practical standpoint of a reasoned approach to the chemotherapy of virus disease is obvious. So without further apology I propose to consider with you some aspects of this important question.

The Sulphonamides and the Antibiotics

The discovery of the sulphonamides and the antibiotics and the demonstration of their efficacy in the treatment of many bacterial infections naturally raised the question of their possible use

in the treatment of virus diseases, and little time was lost in putting this to the test of experiment. Unfortunately many of the earlier trials were ill conceived; the series of cases observed were often much too small and the need for controls overlooked. So it was some little time before the correct answer to this question was obtained, but it has gradually emerged that, so far as what are sometimes referred to as the "typical" viruses are concerned, that is to say excluding the large viruses of the psittacosis-lymphogranuloma group, neither the sulphonamides nor any of the antibiotics so far discovered is either viricidal or viristatic. So uniformly negative have the results been that when a few years back it was claimed that a virus of mouse hepatitis was susceptible to aureomycin and terramycin (Gledhill and Andrewes, 1951) one felt justified in suggesting that a mistake had been made. You will recall that it was thought at that time that two viruses were concerned in the causation of mouse hepatitis, one much more stable than the other, and that it was the labile component which was susceptible to the antibiotics. It has since been shown, however, that the labile partner in the causation of mouse hepatitis is not a virus at all but a protozoan, an eperythrozoon found as a parasite in mouse stocks (Niven, Gledhill, Dick, and Andrewes, 1952). Another finding out of line with the uniform insusceptibility of the viruses to antibiotics is the efficacy of aureomycin and terramycin in "grey lung disease" of mice and cotton rats. This condition is thought to be due to a virus (Andrewes and Glover, 1945), yet not only will these two antibiotics cure this disease in the affected rodents, but in doing so they also eradicate the infective agent. This last fact alone should make one hesitate in accepting the agent of grey lung disease as a virus, because, in those instances where aureomycin and terramycin have been found effective against the large viruses of the psittacosis-lymphogranuloma group, this has been shown to be due to their ability to interfere with the multiplication of the infective agent and not to any direct lethal action; clinical cure leaves a carrier state. There are other features of grey lung disease which make one doubt whether the responsible agent is a virus, and one of them is the complete inability to produce any immunity to the condition. So it may turn out that this remaining exception will disappear and the uniform insusceptibility of the typical viruses to sulphonamides and the antibiotics will in fact be established. This is not to say that the antibiotics or even the sulphonamides are without their uses in the treatment

of disease in man primarily of viral origin, because they can be of value in the control of those secondary bacterial infections which at times complicate the issue with such serious consequences. But against the primary viral component of the disease complex they are powerless.

The Psittacosis-Lymphogranuloma Group of Viruses

As I have already indicated, the agents of the psittacosis-lymphogranuloma group constitute an exception to what I have been saying. Although, like the typical viruses, they require the environment provided by living cells for multiplication and are filterable in the sense that other viruses are, at any rate at some stage of their development, those of them that have been tested have been found to be susceptible, though in varying degree, to the sulphonamides and to most of the antibiotics (Table I). The virus of lymphogranuloma venereum was the first to be shown to be

TABLE I
SUSCEPTIBILITY OF INFECTIVE AGENTS OF PSITTACOSIS-LYMPHOGRANULOMA GROUP TO ANTIBIOTICS

	Sulphonamides	Penicillin	Streptomycin	Chloramphenicol	Aureomycin	Terramycin
L.G.V.	+	±	—	±	++	++
Psittacosis	A few strains +	±	—	±	++	++
Trachoma	+	±	—	+	++	++
Inclusion conjunctivitis	++	+	—	±	+	+
Mouse pneumonitis	++	+	—	±	+	+
Feline pneumonitis	±	±	—	±	++	++

susceptible to the sulphonamides (MacCallum and Findlay, 1938), and, although the newer antibiotics, such as aureomycin and terramycin, are probably more active therapeutically in this disease, some venereologists still give their preference to sulphonamides because with them there is not the risk of masking a concomitant infection with *Tr. pallidum*, and of course they are much less expensive. It would appear, however, that all strains of lymphogranuloma venereum virus are not equally sensitive to the sulpha drugs (Hurst, Peters, and Melvin, 1950), and in the case of psittacosis virus it is the exceptional strain which is susceptible. We had evidence at the London Hospital in 1940 that some strains of psittacosis virus might be sensitive to sulphonamides when on the strength of some experiments I had made in mice, two patients with psittacosis were treated with sulphapyridine with remarkably good results. These were a middle-aged couple who kept

budgerigars and had recently purchased a new pair of birds which fell ill shortly after being installed in their new quarters. The wife was admitted early in the second week of a febrile illness with signs of an atypical pneumonia. She was put on sulphapyridine and in 24 hours was afebrile and much improved; her convalescence was uneventful. Psittacosis virus was isolated from her sputum and she developed psittacosis group antibodies. Her husband, who had been left to look after the birds, sickened in turn and was brought into hospital on the third day of illness. He also was given sulphapyridine with an equally dramatic result. His temperature fell to normal in 24 hours and remained down; recovery was complete and rapid. Despite the early intervention with successful chemotherapy the husband developed psittacosis antibody which, however, did not reach a very high titre, and to complete the investigation psittacosis virus was isolated from the budgerigars. My purpose in mentioning this episode is not to suggest that sulphonamides should be tried in the treatment of psittacosis because, of course, the preparations of choice for this purpose are aureomycin or terramycin; it is only to draw attention to the fact that although the vast majority of strains of psittacosis virus are unaffected by sulphonamides a few undoubtedly are. The classical strain 6 B.C. used by Morgan (1948, 1952a and b) in his studies of the growth requirements of psittacosis virus possesses this characteristic, and other sensitive strains have been encountered in America. In contrast to this the virus of mouse pneumonitis would seem to be uniformly sensitive to the sulphonamides; in fact one profits from this at times to prevent the activation of a latent infection with this virus in mice which are being used for experiment with a sulphonamide-insensitive infective agent: a prophylactic dose of a sulphonamide effectively prevents such a misadventure. Trachoma and inclusion conjunctivitis viruses both appear to be sensitive to sulphonamides, though some authorities still doubt whether the effect of sulphonamides in the former disease is due to their action on the virus rather than on the secondary bacterial invaders. The interesting and important thing about the susceptibility to sulphonamides of the agents of the psittacosis-lymphogranuloma group is that, as in the case of bacteria, the action of these drugs is reversed by P.A.B.A. This has been clearly demonstrated by Morgan (1948) in his experiments with psittacosis virus, including the sensitive strain 6 B.C. already mentioned. He also showed that pteric acid behaved in the same way; both compounds antagonized

the effect of sulphonamides on the growth of this strain of psittacosis virus in a competitive manner. Since pteroyl glutamic acid was also found to antagonize sulphonamides but non-competitively, and glutamic acid was without effect, Morgan suggested that this virus made use of P.A.B.A. to synthesize pteroyl glutamic acid which it requires for growth. Regarding the antibiotics, there is not really very much to say except to draw attention to the inactivity of streptomycin and the superiority of the newer antibiotics aureomycin and terramycin. From a purely academic standpoint, of course, the fact that the infective agents of this group are sensitive to the antibiotics is of no greater significance than their sensitivity to the sulphonamides which we have just been considering; both indicate a degree of independence of the host cell in which they are multiplying which renders them susceptible to attack by these two groups of therapeutic substances. In other words they behave in this respect like bacteria, and, although they have advanced so far in their adaptation to a parasitic mode of existence as to render it impossible for them to multiply without the aid of enzyme systems provided by suitable host cells, they must still possess some metabolic independence. One would expect, too, that when they increase in number they would do so like bacteria by binary fission, and this is borne out not only by the appearance they present in preparations made from the actively multiplying virus, but also by the morphological changes produced under the influence of penicillin. Just as penicillin, when acting on a bacterium such as the gonococcus, will produce giant forms by arresting division, but not at the same time stopping growth of the microbe, so it will when acting on these viruses. Abnormally large forms attributable to the action of penicillin have been observed with the viruses of murine and feline pneumonitis (Weiss, 1950) and with the virus of lymphogranuloma venereum (Hurst, Landquist, Melvin, Peters, Senior, Silk, and Stacey, 1953).

The Possible Mode of Viral Multiplication

The striking contrast between the behaviour of the typical viruses and the agents of the psittacosis-lymphogranuloma group towards sulphonamides and the antibiotics arouses speculation as to its possible significance. One realizes of course that this is one of the reasons for removing the latter group of infective agents from the viruses and classifying them with the rickettsiae, a procedure which has received the sanction of the majority of those who have studied the subject. But does this

necessarily mean that viruses and rickettsiae differ fundamentally in their nature and make use of entirely different procedures for their reproduction? Might it not be that the difference was more apparent than real and that the viruses had merely progressed further on the downward path of adaptation to parasitism? This would be more in keeping with the Laidlaw-Green hypothesis of the nature of viruses, and the increasing dependence on the enzyme systems of the "parasitized" cells would account for the insensitivity of virus to the sulphonamides and antibiotics. The difference between rickettsiae and viruses might be a quantitative rather than a qualitative one. In an attempt to answer these questions I propose to consider with you some of the recent work on the mode of virus multiplication.

Until comparatively recently this aspect of virology had received little attention, and it had been assumed without giving the matter really careful consideration that viruses, like bacteria, multiplied by binary fission. Evidence has been accumulating, however, suggesting that this assumption is incorrect. For one thing it has been claimed that the growth curves of viruses rise in a stepwise manner, and that this is not what one would expect if one virus particle divided to form two, the two then dividing to produce four, and so on by geometrical progression, as is the case with bacteria in the log phase. And then, when infection with a virus is set up, either in tissue culture or in the animal, for a varying period of time before new virus appears it may be difficult, or even impossible, to demonstrate the presence of infective virus, suggesting that multiplication may be occurring in a hitherto unrecognized and non-infective form. It has also been claimed that the influenza viruses incorporate a host tissue component in themselves during the process of reproduction. These and other findings have suggested that viral multiplication proceeds by a process of replication of non-infective subunits followed by their reassembly to form the complete fully infective virus, and the work of Burnet and his colleagues on the formation of hybrid forms of influenza virus (Burnet, 1953) has given support to this conception. But it is the work which has been done in the past 10 years on the mode of reproduction of the bacteriophages, or bacterial viruses as they are sometimes called, which has been largely responsible for the present attitude to the problem of virus multiplication. Rightly or wrongly this work has determined the lines along which the investigation of virus reproduction should proceed, and it has dominated interpretation of the findings. In view of this it would,

I feel, be expedient at this juncture briefly to refer to the salient features of the story of bacteriophage reproduction.

Bacteriophage Reproduction

The bacteriophages, as you know, are filterable agents which parasitize bacteria. Many species exist with a varying range of hosts just as in the case of animal and plant viruses. When infecting a susceptible bacterium they enter and multiply in the bacterial cell, and the usual outcome is destruction of the bacterium by lysis with the liberation of a greatly increased number of bacteriophage particles. Most authorities hold the view that bacteriophages belong to the order *Virales*, and, since they and their bacterial hosts are so easily and inexpensively maintained in the laboratory, they have been used extensively in the study of the host-virus relationship. It is for this reason that some of the first attempts to solve the problem of virus reproduction have been made with the bacteriophage. The exact time of infection and the extent of that infection can be determined with accuracy, and, since the completion of the reproductive cycle ends in lysis of the bacterium with the liberation of the newly formed bacteriophage, the exact time occupied by the reproductive cycle—the "burst" time—can be ascertained and its product measured. It seems clear that it is in the interior of the bacterium that the bacteriophage is multiplying, since neither dark-ground nor electron microscopy reveal anything taking place at the surface of the bacterium and the multiplying phage is not accessible to the action of phage antiserum. But it has proved quite impossible by any form of microscopy to determine what is occurring inside the infected bacterium, and in order to find out means have been evolved for breaking open the bacterial cells and examining their contents at intervals after infection by microscopical, serological, and chemical methods as well as by tests for infectivity.

Briefly the story revealed is as follows (Fig. 1). The time lapse between infection and lysis of a bacterium is a matter of minutes, from 12 to 60 according to the species of phage. From the inception of infection to midway in the cycle it has proved impossible to recover infective phage; the phage is described as being in eclipse or to be passing through a dark period. It is somewhere about mid-cycle that mature virulent phage reappears at first in small numbers but soon in rapidly increasing quantity until lysis occurs and the new phage particles are liberated. What is happening during this period of eclipse? Microscopy, even electron

microscopy, has proved of no assistance here. Chemical investigation tells us that the reappearance of infective phage coincides with an abnormal synthesis of desoxyribonucleic acid. The com-

tence of a non-infective phase as a necessary step in multiplication. The idea of an eclipse phase permeates all thought on virus multiplication; to me it appears to have become an obsession.

		Test of Contents of Bacteria for :		
		Lytic Phage	C.F.T. Antigen	Blocking of Neutralizing Antibody
Single cycle of phage multiplication

—	—	+
	±	±	+
	+	+	+

FIG. 1.—Schematic representation of bacteriophage multiplication.

plement-fixation test made with the appropriate phage antiserum remains negative until mature infective phage reappears (Rountree, 1951). The only evidence that phage material is present in the infected bacterium during the eclipse is that the bacterial contents liberated during this period have the power specifically of blocking the neutralizing power of the appropriate autophage serum (Luria, 1953). And the particle size of this material is less than that of mature phage since it goes through collodion filters which retain the phage. The hypothesis put forward to explain these happenings supposes that the infecting phage, having entered a bacterium, breaks up into smaller non-infectious particles which, with the aid of enzyme systems of the bacterial cell, multiply by a process of replication. Subsequently these small, non-infective particles or sub-units are reassembled to form complete infective bacteriophage. Were I concerned with a critical appraisal of the work on bacteriophage multiplication there is a vast amount of additional evidence that I should have to produce, and that most interesting and brilliant work of Lwoff and his co-workers on the incomplete form of the bacteriophage, or "prophage" as he terms it, would call for particular mention. But my concern this afternoon with bacteriophage multiplication is the influence which the views on this problem have had on the question of virus multiplication, and for this it suffices to present the matter in its briefest outline, drawing attention to its essential feature: the eclipse and the exis-

Virus Multiplication

Psittacosis-Lymphogranuloma Group.—We

have already seen that the available evidence points to binary fission as the mode of reproduction used by these agents, and I would not have referred to them again were it not that Sigel and his colleagues Girardi and Allen (Sigel, Girardi, and Allen, 1951; Girardi, Allen, and Sigel, 1952) have recently advanced the view that a non-infective phase is an essential component in the cycle of multiplication of meningo-pneumonitis virus, which is one of the psittacosis group. They made use of a strain of virus adapted to growth in the allantoic sac of the developing hen's egg, and they found that when injected by this route the virus was slowly taken up by the cells in the chorio-allantois, reaching a peak by

about the fourth hour (Fig. 2, lower curve). Subsequently, infective virus in the membranes gradually decreased in quantity until about the nineteenth hour when little or none could be recovered. This was followed by its reappearance, the titre of infective virus increasing slowly at first and then more rapidly to reach a peak about the forty-eighth hour. The same shape of growth curve was obtained if the membranes were removed at the fourth hour and, after washing to remove unabsorbed virus, used for setting up tissue cultures in which the cycle of multiplication was allowed to run to completion. Since infective virus was not lost from the membranes to the surrounding fluid in these cultures during the "eclipse" period, it was argued that the fall in titre of infective virus in the membranes was due to a change to a non-infective form. And, since cultures set up with membranes removed from the eggs at different times during the eclipse and therefore containing varying amounts of infective virus, all reached approximately the same titre at 48 hours (Fig. 2, upper curve), it was concluded that the newly formed virus must have been derived from a non-infective precursor. This conclusion implied that, as in the case of the bacteriophage, the newly formed infective virus was made by the reassembly of non-infective sub-units, but one would have thought that had this been so the growth curve would have risen much more steeply from the twentieth hour onwards, whereas the rate at which it rose hardly exceeded the rate of fall from the fourth to

FIG. 2.—Growth curve of meningo-pneumonia virus in allantoic membrane *in ovo* (lower curve) and potential for virus development of allantoic membranes harvested during the latent period (upper curve). (From Girardi, Allen, and Sigel, 1952.)

[By courtesy of The Journal of Experimental Medicine.]

the nineteenth hour. And then the idea of an agent of the psittacosis group multiplying in this way seemed out of line with earlier observations on the cycle of morphological change which these micro-organisms undergo when multiplying (Bedson and Bland, 1932). So Gostling and I (Bedson and Gostling, 1954) have examined this matter. We used a strain of psittacosis highly virulent for the mouse, giving the inoculum by the peritoneal route and following the development in the spleens of mice removed at intervals after inoculation and washed thoroughly to remove any of the infective agent contaminating the outside of the spleen. Suspensions of the washed spleens were examined not only for their infectivity, but also by the complement-fixation test for antigenic material both formed or unformed, and in addition smears were made and stained for microscopical examination. The level of infectivity remained more or less unchanged from one hour after inoculation until the twelfth hour, but had started to rise steeply by the sixteenth hour and continued to do so until the forty-eighth hour, when the animals were moribund (Table II). With the microscope none of the infective agent could be seen during the first seven hours, presumably because it was present as elementary bodies, the form in which it was introduced, and these cannot be identified with any certainty unless in considerable aggregates. About the eighth hour, however, occasional large forms or initial bodies could be detected occurring singly and in pairs, and at the tenth and twelfth hours they were more numerous, forming at times small clumps. By the sixteenth hour the infective agent was still exclusively in the large form, but these

were now much more numerous, forming clumps of considerable size, and many dividing forms were readily found, as was the case in the earlier preparations. The 48-hour preparations showed little else but elementary bodies in enormous numbers. The complement-fixation test made at intervals after infection failed to reveal any specific material which had escaped detection by infectivity test or microscopy. The interpretation we put on these findings is that the agent of psittacosis when multiplying in the mouse spleen does so by binary fission. Introduced into the peritoneal cavity in the form of elementary bodies, the mature infective form, a small number travel to the spleen and, having entered suitable cells, spend the next few hours with the help of the enzyme systems of the parasitized cells building themselves up and

TABLE II

INFECTIVITY OF MOUSE SPLEENS COLLECTED FROM ONE TO 16 HOURS AFTER INOCULATION (FROM BEDSON AND GOSTLING, 1954)

Time after Inoculation of Collection of Spleen	Results in Individual Mice (Time to Death in Days)	Mortality (Dead/Inoculated)	Average Time to Death (Days)
1 hour ..	6, 7 4, 6, 6, 6, 7, 8 8, 8, 8, 8, 8, 9, 9, 9, 9, 10, S	19/20	7.5
4 hours ..	6, 7, 7, 7, 7, 12 8, 8, 9, 9, 11, S	11/12	7.6
5 " ..	7, 9, 9, 9 7, 7, 7, 7 9, 10, 10, 11	12/12	8.5
6 " ..	6, 7 6, 6, 6, 6, 7, 7	8/8	6.5
8 " ..	7, 8, 10, 11 7, 7, 7, 8, 8, S	9/10	8.1
10 " ..	6, 7, 7, 7 8, 8, 10, 10	8/8	7.9
12 " ..	7, 7, 7, S	3/4	7.0
16 " ..	4, 4 5, 6, 6, 6 4, 4, 5, 6 5, 5, 6, 6 2, 2, 2, 2 2, 2, 2, 2 2, 2, 2, 2 2, 2, 2, 2 2, 2, 2, 2 2, 2, 2, 2	16/16	5.0
48 " ..	2, 2, 2, 2 2, 2, 2, 2 2, 2, 2, 2 2, 2, 2, 2 2, 2, 2, 2 2, 2, 2, 2 2, 2, 2, 2 2, 2, 2, 2	18/18	2.0

Inoculum was 0.5 ml. intraperitoneally of a 5% w/v suspension of spleen in broth.

acquiring the energy necessary for division. This constitutes a latent or lag period which has as its visible end product the large form or initial body. Multiplication of the agent now enters the logarithmic phase, the large forms dividing and increasing in number at first slowly but soon more rapidly. From the sixteenth hour onward the individual infective particles become progressively smaller with each successive division so that by the forty-eighth hour they are all in the small elementary body form once more; a cycle of multiplication has been completed in which a non-infective form seems to play no part. It is true that the infective agents of the psittacosis-lymphogranuloma group have by more or less general consent been removed from the *virales* and put with the *rickettsiae*, so that one might expect them to reproduce in this orthodox way. The interesting thing, however, is that as little as two years ago evidence was being produced to show that they passed through an eclipse phase when multiplying in which a non-infective form played an essential part—in other words, that their mode of reproduction was bacteriophage-like.

The Influenza Virus.—One can safely say, I think, that no animal viruses have been more extensively studied from the point of view of their mode of multiplication than the influenza viruses. And I think it is equally true to say that in the opinion of the majority of virologists the available evidence strongly suggests, if it does not prove, that the influenza viruses, like the bacteriophage, multiply by replication with subsequent reassembly of non-infective sub-units. There are four main points on which this thesis is built: (1) The step-wise nature of the growth curve; (2) the occurrence of an eclipse of infective virus in the early hours of reproduction; (3) the independent appearance of the component parts of the virus; (4) the inclusion of host material in the virus particle.

May I consider these points in turn with you? It was Hoyle, I think, who first drew attention to the fact that the growth curve of influenza A virus was step-wise and not exponential, and the same has been shown to be the case with other animal viruses such as herpes (Modi and Tobin, 1954), mouse-pox (Nossal and de Burgh, 1953) and pneumonia virus of mice (Ginsberg and Horsfall, 1951) and others. When influenza virus is introduced into the allantoic cavity of the embryonated egg it rapidly disappears from the allantoic fluid, being taken up presumably by the allantoic cells, and by the end of the first hour from 70 to 90% of the inoculum has disappeared. New infective virus does not appear in the allantoic fluid until some six hours after infection, but when it does

so it rapidly increases in quantity, reaching a peak about the eighth hour (Fig. 3). For the next few hours no further new virus appears in the allantoic fluid, but after this interval the titre rises again

FIG. 3.—Step-wise increase in virus content of allantoic fluid of egg inoculated with influenza A virus. (From Hoyle, 1948.)

[By courtesy of The British Journal of Experimental Pathology.]

steeply. This step-wise increase in virus has been taken to indicate that the virus is not multiplying by binary fission but in a cyclical manner, each cycle in the case of influenza A virus occupying about eight hours. In criticism of this interpretation it has been argued that the step-wise nature of the curve merely reflected the intracellular habit of growth of the virus and was due to the breakdown of the infected cells with the liberation of their viral content at the end of a cycle. This argument may possibly lose some of its force should it prove, as it is now claimed, that breakdown of the cell is not essential for liberation of the contained virus. And Hoyle's (1950) claim that the complete influenza virus is formed at the surface of the infected cells, which is supported by Wyckoff's findings with the electron microscope (1951), suggests that there may be something in this idea. It is difficult, therefore, to decide at this juncture whether or not the peculiar shape of the growth curve is inconsistent with a virus multiplying by binary fission. The so-called eclipse, when for a time after infection little or no infective virus can be recovered from the cells which the virus has entered, is an essential feature of the modern conception of virus multiplication; it is during this period that replication of non-infective sub-units is thought to take place. In the case of influenza virus this phase in its development has been intensively studied, and Hoyle (1953) in this country has played a leading part in this work and

in the interpretation of the facts which it has brought to light. There is a series of tests, in addition to that for infective virus, which can be used for this purpose. The so-called soluble or S antigen, which is smaller in size than the virus particle and occurs separately from it, can be detected by the complement-fixation test. Influenza virus agglutinates the red cells of certain species and will still do so when it has been rendered non-infective by certain means; inactive virus will also interfere with the multiplication of homologous virus. The specific or V antigens of the influenza virus will also fix complement with influenza antisera. Hoyle has made use of all these tests in investigating the eclipse phase. We have seen already that only a small fraction of the infecting virus which disappears from the allantoic fluid and passes presumably into the chorio-allantoic membranes can be recovered from them and that infective virus does not reappear in the fluid until about the seventh or eighth hour after infection at the completion of the cycle. Hoyle (1952) finds that soluble antigen makes its appearance in the membranes about the end of the second hour and that haemagglutinin can be extracted from them one or two hours later (Fig. 4). About this time—the end of the fourth and beginning of the fifth hour—the complement-fixation test for the specific antigen becomes positive, and shortly after this the com-

FIG. 4.—Recovery of agglutinin and complement-fixing antigens from the chorio-allantoic membrane of eggs inoculated with influenza A virus in terms of the amounts in the virus originally entering the cells. (From Hoyle, 1952.)

[By courtesy of The Journal of Pathology and Bacteriology.]

plete, fully infective virus makes its appearance. Hoyle believes that the virus on entering and infecting cells breaks up into non-infective sub-units which he identifies with the soluble antigen. These sub-units multiply by a process of replication and are then reassembled to produce first of all the incomplete haemagglutinating and interfering virus which, with some finishing touches, becomes the complete infective virus. Not all those who have studied the reproduction of influenza virus are prepared to accept Hoyle's thesis, and Henle (1953), in whose department so much interesting research on this problem has been done, concludes in a recent review of the subject that the difference in the temporal relationship of the development of the various viral properties is probably more apparent than real, and that the soluble antigen is more likely a product of host-virus interaction than a building block of the virus particle. I should perhaps hasten to add that Henle, none the less, inclines to the conception of the formation of new virus in stages with replication of a non-infective particle an early and important one. The fourth point supporting the thesis of replication in sub-units during the eclipse period that I wish to consider with you concerns the presence of host material in the influenza virus particle. At first thought to be a contamination of the virus particles it now appears that this material might be an integral part of the virus. In support of this view is the work of Knight (1946), and, more recently, the findings of Wilson Smith, Belyavin, and Sheffield (1955). The latter investigators have shown that purification by repeated adsorption on to and elution from red cells does not vary the proportion of host material, and that in order to make this material fully available for serology heating the virus at 60° C. is necessary. This has suggested to them that the host material is situated deeply in the virus particle, the heating being necessary to destroy heat-labile surface antigenic material masking it. Now the serological evidence that host material is present in the influenza virus particle is that when the virus is grown in the hen's egg it fixes complement with an antiserum made against normal chick embryo membranes (C.A.M.) and when grown in mouse lung with an anti-mouse lung serum. Wilson Smith and his colleagues have shown, however, that C.A.M. antiserum not only fixes complement with influenza virus grown in the egg but also with virus grown in mouse lung, and that the reason for this anomaly is that the host material antigen in the influenza viruses are of the Forsmann type, and both mouse tissues and chick embryo membranes possess Forsmann antigen.

They have also adduced evidence to show that this Forsmann-type antigen in the influenza virus differs from the dominant Forsmann antigen of the chorio-allantoic membrane, and they consider that this is due to the modification which the host material undergoes when it is incorporated in the virus particle. But it might be that influenza virus itself made Forsmann antigen just as some micro-organisms such as the pneumococcus do, and that cross-reactions between host material antisera and influenza virus was due to this and not to the incorporation of host material. One would like to know how influenza virus grown in a host not possessing Forsmann antigen behaved, for without this knowledge it could be argued that the only reason for identifying the Forsmann-type antigen of the virus with host material was that the virus multiplied by the reassembly of replicated sub-units; one would be turning in a circle!

Observations on the Eclipse Phenomenon

It would be quite impossible in the time at my disposal adequately to present the evidence supporting the present conception of virus multiplication, so that I have contented myself with giving what I consider to be the salient points, citing in their support the minimum of evidence. And in doing so I realize that I may have done injustice to some virologists either by an inadequate presentation of their work or by omitting to mention it. Should I have been guilty of misrepresentation I would claim that it has been from inadvertence and not intent. My intention has not been to attempt to prove or disprove the conception of virus multiplication to which so many virus experts to-day subscribe, but to point out that the concept is still in the realm of hypothesis and to suggest that it may possibly be wrong. For too long, in my opinion, both thought and experimentation on the subject of virus multiplication have been coloured by the work which has been done with the bacteriophage; the shadow of the eclipse has lain heavily on it. I would like to make a plea for a new approach to this problem of virus multiplication, putting aside all preconception and the mesmeric influence of the word eclipse and all that it implies. For instance, might it not be possible that the so-called eclipse is no more than a latent or lag period in which the virus is adjusting itself to its new environment in preparation for multiplication and during which, according to the nature and suitability of the environment, a varying portion of the seeded virus becomes inactive, having failed to make the grade? There is some

evidence which provides support for such an explanation. A few years ago Davenport and Francis (1951) published their findings in a study of the multiplication of influenza virus in the mouse lung. They used strains of both influenza A and B virus, and they compared the rate of multiplication of mouse-adapted and unadapted strains (Figs. 5 and 6). With the former, the virus got off the mark with little or no hesitation, and by the sixth hour the curve of infective virus had risen appreciably above zero level and continued to do so to reach a peak well above that attained by the unadapted strains. What was more, with the unadapted strains there was an initial drop in the amount of infective virus recoverable from the lungs which reached a trough about the sixth hour. It was the poor multipliers, the strains unadapted to growth in the mouse lung, which showed an eclipse. It might, of course, be argued that owing to the absence of observations between zero hour and the sixth hour the eclipse had been missed in the case of the mouse-adapted strains, but from the way the growth curve is rising at the sixth hour this seems highly problematical. And I am quite certain what explanation would be put on these findings if the infective agents in question had been bacteria! The authors themselves conclude that whatever explanation one offered of the different

FIG. 5.—Rate of multiplication of adapted and unadapted lines of a Type B strain (Warner) of influenza virus in mouse lung. (From Davenport and Francis, 1951.)

[By courtesy of The Journal of Experimental Medicine.]

FIG. 6.—Rate of multiplication of adapted and unadapted lines of a Type A—Prime strain (Rhodes) of influenza virus in mouse lung. (From Davenport and Francis, 1951.)

[By courtesy of The Journal of Experimental Medicine.

rates of multiplication of the adapted and unadapted strains one thing was abundantly clear: the adapted strains were much the better parasites. I would suggest that studies with strains of virus adapted and unadapted to a given cultural environment and paying particular attention to the early hours of multiplication might be rewarding. In the case of herpes virus also there is an indication that the eclipse is no more than a lag period in which varying amounts of virus die by the way. It has been shown that when herpes virus is grown on the chorio-allantois (Scott, Coriell, Blank, and Gray, 1953; Modi and Tobin, 1954) or in tissue culture (Modi and Tobin, 1954) a very large part of the virus taken up by the cells ceases to be detectable as infective virus, and it has been suggested or implied that this disappearance is due to a change to a non-infective vegetative form in which the virus is multiplying; it passes through an eclipse phase. Gostling and I have found, however, that in cultures of herpes virus made with either chick embryo mince or single cells which had been infected in the cold, washed to remove unattached virus, and then put up in a medium containing sufficient antibody to neutralize all the virus present, infective virus can be demonstrated in the

cells at zero hours as well as after three and six hours' incubation at 37° C. And although in these experiments a progressive fall in the amount of infective virus did occur in the first six hours of cultivation, the growth curves obtained by no means gave unequivocal support to an eclipse as an essential feature in the multiplication of herpes virus.

The Bearing of the Mode of Virus Multiplication on Chemotherapeutic Possibilities

Is all this of no more than academic interest? Or will a knowledge of the way in which viruses multiply and of their growth requirements provide us with a rational approach to the problem of the chemotherapy of virus infections? There are some who are doubtful about this, and in a recent review of the chemotherapy of virus diseases Hurst (1953) expresses the view that more rapid progress might well be achieved by the partially empirical screening of compounds in the mouse and following up the leads so obtained. He points to the relative ease with which virus multiplication in tissue culture or *in ovo* can be interrupted and how rarely substances active in this way have been found to have any protective action at all in the animal. And in support of the former approach to the problem one can cite the discovery (Hurst, Melvin, and Peters, 1952) of the quite remarkable therapeutic activity of mepacrine in equine encephalomyelitis and rift valley fever in the mouse. Incidentally this substance proved quite inactive against equine encephalomyelitis in the chick embryo. However, Hurst (1952) lists a number of substances which have been found to inhibit virus growth in tissue culture, some of which have not been without some action in the animal. It seems to me, therefore, that with a more exact knowledge of the way in which viruses multiply in the cell and of their metabolic requirements, the possibility of interfering with their growth cycle by means of biological and other agents would be brought nearer realization. And it seems to me also that, should it prove that some of the typical viruses reproduce by binary fission and not by replication of non-infective sub-units, the chance of finding effective chemotherapeutic procedures against them would be to that extent brighter, since it would suggest that they had not so completely identified themselves with the parasitized cells. The truth will emerge in time, and for the moment signs are not wanting that chemotherapeutic measures against virus infections are within the bounds of possibility.

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