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ASPECTS OF THE PATHOGENESIS OF VIRUS DISEASES

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INTRODUCTION

In the past, there have been two principal methods available for the study of the pathogenesis of virus diseases. In the first method, organs and tissues from infected animals were sectioned and examined by routine histological techniques. Many valuable observations were made about the organs affected and the type of lesions produced, but it was often difficult to know exactly which cells were infected except when typical inclusion bodies were present. Often the studies were limited to material taken very late in the disease or after death, so that little was learned about the spread of infection through the body and the development of lesions.

Fenner's work on ectromelia infection in mice (55) is the classical example of the second type of study of the pathogenesis of virus diseases. Using what may be called the routine titration approach, he assayed organ and tissue suspensions at different times after infection, and was able to analyze the events which preceded the outward signs of disease. Fenner focused attention on the incubation period in virus-infected animals;

clearly an understanding of this incubation or eclipse period is necessary if the disease process is to be understood, just as an understanding of the eclipse period in virus-infected cells is necessary if the infection of cells is to be understood. With the limited techniques available, however, Fenner could do no more than follow the growth of virus in the main organs and tissues of the body. The infectious process could only be described in terms of virus growth curves, and for a given organ nothing could be discovered about the site of initiation of infection, the spread of infection, or the types of cell supporting virus growth.

This basic limitation in studies of the pathogenesis of virus diseases lasted until the development of the fluorescent-antibody technique. When this powerful and important technique became available, individual infected cells could be identified with confidence in tissues, and at last the process of infection could be analyzed histologically. A fresh approach could now be made to old problems, like the growth of viruses in nervous tissue or capillary endothelium. Unfortunately, in spite of the power and promise of this new approach, it has not often been used in the study of the pathogenesis of virus diseases. This is partly due to certain limitations which still make the technique unsuitable for tissues infected with some viruses, but, even in the virus diseases where it can be profitably used, investigators have too often been content with a glimpse of infected tissues right at the end of the disease process. One other reason why the possibilities of the fluorescent-antibody technique have not been fully exploited is that many virologists have, in the past 10 years, turned to tissue culture systems. The possibilities of the routine titration approach to studies on infected animals had been largely exhausted, and it was perhaps a relief to turn from the horrifying complexity of infected animals to the cleaner, simpler, and more readily controllable in vitro systems. This boom in tissue culture meant that cell-virus interactions were studied at the expense of host-parasite interactions. Furthermore, as the vaccines produced by tissue culture research make the important virus diseases one by one unimportant, so an understanding of these diseases becomes less necessary from a practical point of view. Smallpox and poliomyelitis may even disappear as human diseases before a very thorough knowledge of their pathogenesis has been attained.

In this review, a survey of certain aspects of virus infections at the host-parasite level is made, to draw attention to the power of the fluorescent-antibody technique in studying these infections. Although some of the neglected aspects in the pathogenesis of virus infections will be dealt with, no attempt has been made to review the subject in a general way. Inevitably, much of the experimental data comes from the author's own laboratory and concerns the poxvirus infections in which the fluorescent-antibody technique can be used to analyze events in terms of individual cells. In many of the studies to be described and reviewed, it became apparent that macrophages are often of key importance in infected animals, and the first part of the review will, therefore, deal with the role of macrophages in virus infections. The action of these cells in clearing virus particles from the blood stream will be described, and this will be followed by an account of virus infections of the liver and the different types of virus-macrophage interaction seen in this organ. The role of macrophages in determining the susceptibility of animals to infection will be referred to, as well as the influence of other microorganisms on macrophages and thus on virus diseases. The neglected subject of viremia is then discussed, and viremias are classified in relation to the clearance of virus from the blood by macrophages. The next section is devoted to the role of capillary endothelium in virus infections, again in relation to clearance by macrophages. Present knowledge about lymphoid tissue in virus infections is then surveyed at some length, and the subject of the immune response is discussed. Subsequent sections deal with virus infections of the peritoneal cavity, connective tissues, bone marrow, cerebrospinal fluid, lung and alimentary canal. There is a final short section saying something about the role of microphages (polymorphs) in virus infections. The discussion is devoted to a further consideration of virus-macrophage interactions, and of the general subject of phagocytosis in virus diseases.

MACROPHAGES IN VIRUS INFECTIONS

Macrophages are the rather large mononuclear cells, scattered through different tissues, which are capable of taking up foreign particulate material, as well as certain dyes. Metchnikoff

(100) was the first to distinguish these cells from the microphages, or polymorphonuclear leukocytes, whose small size, elongated nucleus, chemotactic powers, and rapid production rate can be considered as specific adaptations for very rapid mobilization and diapedesis into acutely inflamed tissues. The reticuloendothelial cells are macrophages, and these were described as an anatomical and physiological unit by Aschoff and Kiyono (4). The ability to take up foreign particulate material, however, is not an exclusive prerogative of these cells; ordinary capillary endothelial cells, for instance, may do so under certain conditions (2, 16). Indeed, this capacity of cells to ingest foreign materials is perhaps a very primitive one, and a large variety of cells may display phagocytic powers (74, 116, 143). Nevertheless, certain cells have become specialists, so that one of their principal functions in the body is the ingestion, intracellular digestion, and disposal of foreign material. "Foreign material" is taken to include effete host cells or host cell debris, as well as foreign inert particles and microorganisms. Macrophages may have additional functions, but this "scavenger" function is the principal one which will be considered in relation to virus infections.

Macrophages are situated in all the major compartments of the body. Those lining the liver, spleen, and bone marrow sinusoids monitor the blood and remove, for instance, circulating effete red blood cells. Those lining the sinuses of lymph nodes monitor the lymph, removing microorganisms or inert particles brought in the afferent lymph stream. Those lining the pleural and peritoneal cavities monitor these cavities, and those lining the respiratory tract monitor the respiratory fluid film, ingesting inhaled dust particles, mineral particles, or microorganisms. Finally, large numbers of macrophages live in, move through, and monitor the connective tissue spaces throughout the body. Extravasated blood or invading microorganisms are removed, and large masses of host cells may be phagocytosed and digested during the repair of damaged tissues or the involution of embryonic structures.

Virus particles, it is suggested, tend to be taken up by macrophages as are other foreign particles. Since these cells effectively monitor the main body compartments, they inevitably encounter infecting virus particles, probably at an early stage of the infection. On the results of this virus-macrophage encounter, the course of the infection can depend.

Much of the available information concerning phagocytosis by macrophages comes from experiments on the clearance from the blood of intravenously injected particles, and the first part of this section deals with this sort of experiment in relation to virus infections. This is followed by an account of virus-macrophage interactions in the liver, where macrophages, in monitoring the blood, have an opportunity to control the entry of virus to this organ. It will be seen that the course of the infection can be determined by these strategically placed liver macrophages. In subsequent parts of this section, other aspects of the behavior of macrophages in virus infections are discussed. Macrophages also figure prominently in other sections of this review, and the general subject of the phagocytosis of virus particles is dealt with in the discussion.

Blood Clearance Studies

Reticuloendothelial macrophages, particularly those in the liver, are capable of clearing the bloodstream of intravenously injected particles, and numerous studies of this process of clearance have been made. The particulate and colloidal preparations studied include bacteria (23), fat droplets (82), albumin-globulin complexes and saccharated iron oxide particles (15), colloidal chromium phosphate particles (43), and carbon particles (20). In many cases, the cleared material has been identified in macrophages (17, 82, 119). A number of factors have been shown to influence the rate of clearance, and these were first studied quantitatively by Benacerraf and his collaborators. Particle size is important, and it has been shown that larger particles tend to be cleared more rapidly than smaller ones (42, 170). The dose also influences the clearance rate. Below a certain critical dose, particles may be cleared completely on a single passage through the liver, and indeed clearance rates have been used to measure liver blood flow (43); with larger doses, the clearance rate is inversely proportional to the dose (20). Finally, opsonins are probably of fundamental importance in clearance, and adequate opsonization of particles is perhaps always necessary. Jenkin and Rowley (83) showed this to be true for the clearance in mice of so-called inert carbon particles.

The clearance of viruses from the blood has

also been investigated, but to a more limited extent. In most of the studies with nonviral materials, mice have been used, and the viruses whose clearance curves have been studied in mice include T_7 bacteriophage, poliomyelitis, Semliki Forest, and vaccinia viruses (Mims, *unpublished data*), Rift Valley fever (RVF) virus (102), ectromelia virus (106), and Newcastle disease virus (NDV) and vesicular stomatitis viruses (28). In some cases, the cleared virus has been demonstrated in liver macrophages (28, 52, 106, 110). Representative virus clearance curves are shown in Fig. 1, and it can be seen that particle size affects clearance rate in the same way as has been reported for nonviral materials. Larger viruses, like vaccinia or vesicular stomatitis, are cleared much more rapidly than are smaller viruses. The effect of particle size may be no more than a general trend, because small particles, for instance, could clump to form larger ones when introduced into the blood, and, in any case, clearance must depend to some extent on the nature of the particle.

Another feature of virus clearance curves is that clearance does not continue to occur at the same rate, and the curve flattens out, leaving an "uncleared tail." Whatever the amount of virus introduced, a constant proportion remains after clearance (103). This "uncleared tail," also seen in experiments with inert particles, has in some cases been shown to be due to a small fraction of

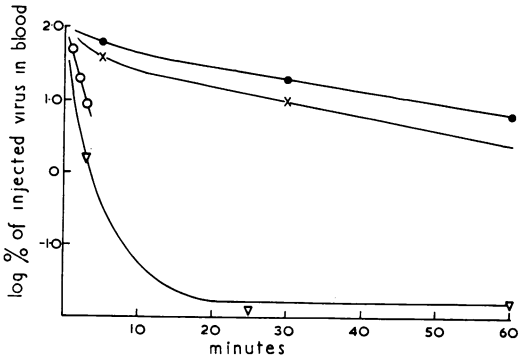

FIG. 1. *Virus clearance curves in mice. The viruses, types of virus preparation, and approximate sizes were as follows:* ●, *T_7 bacteriophage, purified preparation, 30 mμ (Mims, unpublished data);* ×, *Rift Valley fever, infective mouse serum preparation, 30 mμ* (102); ○, *vesicular stomatitis, purified P^{32}-labeled preparation, 90 mμ (28); and* ▽, *vaccinia, partially purified preparation, 250 mμ (Mims, unpublished data).*

smaller, less rapidly cleared particles present in the inoculum (42). One important explanation of the uncleared tail in virus clearance studies is that some of the particles become associated with blood cells, as has been shown for ectromelia virus (106) and gold colloids (144), and are therefore not removed by reticuloendothelial macrophages. In such cases, the uncleared material is not cleared when injected into another mouse. Usually, however, the uncleared virus is in the plasma and is cleared normally when injected into a fresh mouse, as was shown for RVF virus (103). Conceivably, the recipient mouse clears the uncleared virus only because the virus particles are coated with foreign plasma proteins from the donor mouse. This possibility was excluded when it was found with Semliki Forest virus that the uncleared tail from an inbred strain of mice (Bagg) was still cleared when injected into a litter mate of the same strain. In this experiment, any plasma proteins which might have coated virus particles would have been immunologically acceptable, but the uncleared virus was cleared in spite of this. A depletion of plasma opsonins would seem to be excluded as a cause of the uncleared tail, at least in the case of Semliki Forest virus, because further injections of the same preparations are cleared down to the level of the uncleared tail (Mims, *unpublished data*). It would seem that the only way of accounting for the uncleared tail is to assume that the uptake of particles by reticuloendothelial macrophages is partly reversible, an equilibrium being reached between circulating and phagocytosed particles. This would explain why a constant proportion of the inoculated particles is uncleared (103). If it is hard to imagine that macrophage phagocytosis is partly reversible, it should be remembered that the phagocytosis of bacteria by monocytes can be reversible, ingestion and egestion being observed in the same cell (164).

Liver macrophages are quantitatively the most important cells responsible for the removal of intravenously injected particles, viral or nonviral. These macrophages, moreover, are morphologically readily distinguishable from the neighboring hepatic cells. For these reasons, virus-macrophage interactions in the liver have been particularly well studied, and virus infections of the liver will now be discussed with special reference to these interactions.

Virus Infections of the Liver and the Importance of Virus-Macrophage Interactions

Viruses infecting the liver usually reach this organ by way of the blood. Since liver macrophages take up virus particles from the blood, this uptake becomes an inevitable first-step in the infection of the liver. In mice, the littoral cells of the liver sinusoids constitute a lining which is, functionally, almost complete, and probably no particle in a sinusoid has access to a hepatic cell except through a monitoring macrophage. Small fenestrations or gaps can be seen by electron microscopy in or between littoral cells, however perfect the fixation, but these are temporary gaps in a living cytoplasmic sheet (160), and it seems unlikely that particles pass through them very easily. Thus, the clearance mechanisms described above ensure not only that circulating virus particles, pathogenic or nonpathogenic, are phagocytosed by liver macrophages, but also that infection of hepatic cells can only be achieved after particles have in some way passed through the macrophage lining of sinusoids. In studies of the growth of viruses in the liver, much has been learned about the types of virus-macrophage interaction which may occur in infected animals; all possible types of interaction have been observed, and these will now be dealt with separately.

No uptake by macrophages. Poliovirus type I, unlike types II and III, is not cleared from the blood when injected intravenously into mice. The fall in blood titer, even after several days, is attributable to thermal inactivation (Mims and Howes, *unpublished data*). This appears to be an unusual instance of failure to clear a foreign particle. Clearance of infectious type I poliovirus occurs in rats, and also in cynomolgus monkeys, which are susceptible to infection. It should be pointed out that the infectious particles assayed in these experiments constitute only a small fraction of the total number (139). Perhaps the noninfectious particles, which are mostly C rather than D antigenically (26, 132), are cleared in mice. There must be a mechanism by which these antigens enter cells, because mice in fact do develop antibodies to infectious virus after large intravenous injections of type I poliovirus. Although the failure of clearance in mice might be attributed to a lack of blood opsonins, the results are the same when virus has been preincubated in an excess of normal cynomolgus monkey serum before injection into mice. Any opsonins present in monkey serum, therefore, do not affect clearance in mice. Alternatively, it could be that mice regard infectious poliovirus type I as self rather than foreign, and attempts were made to see whether this virus is antigenically similar to normal mouse body components. No evidence for this possibility could be obtained. Antibodies to normal mouse spleen and blood cells and to mouse tissue homogenates were produced in rabbits, but they neither neutralized virus in plaque assays nor influenced the clearance of virus in mice.

Most of these clearance experiments were done with large inocula of poliovirus [10^7 to 10^8 plaque-forming units (pfu)]. When one-hundredth of this amount of virus was injected, however, a significant fall in blood titer was detected within the first 2 hr, representing a clearance of more than half the injected virus; there was no further fall in titer within the next few hours. Perhaps with smaller doses of virus there is clearance, but with a high degree of reversibility (see above), whereas with large doses a shortage of opsonins prevents clearance from occurring on a detectable scale.

Uptake and digestion by macrophages. When nonpathogenic viruses are injected intravenously, they are cleared by liver macrophages, and subsequently digested and degraded. The fate of the virus particles can be studied, in conjunction with clearance curves, by use of the fluorescent-antibody technique. The CL strain of vaccinia, for instance, can be seen as antigen in liver macrophages a few minutes after the intravenous injection of large doses of virus. Within 1 hr, this antigen has faded to the vanishing point, and it fails to reappear. This is interpreted as an intracellular digestive destruction of virus particles. From similar observations, it is concluded that intravenously injected influenza virus (subtoxic doses) and myxoma virus in the mouse, and also ectromelia virus in the rat, are dealt with in the same way. Probably most nonpathogenic viruses are largely taken up and destroyed by macrophages in this fashion.

There are good reasons for believing that some viruses, although they are taken up and destroyed by liver macrophages after intravenous injection, are nevertheless capable of infecting hepatic cells. This inherent susceptibility of hepatic cells can be demonstrated as follows. The CL strain of vaccinia is taken up and

destroyed by liver macrophages after intravenous injection into mice, but if large doses are injected up the common bile duct, and thus directly into a number of periportal hepatic cells (110), there is growth of virus in these cells as judged by the reappearance of large amounts of antigen in them after 10 to 18 hr. Influenza virus and myxoma virus in mice, and also ectromelia virus in the rat, behave in this way. It is obviously unnatural to infect hepatic cells by injecting viruses up the bile duct, a procedure which literally flushes the inoculum through the cytoplasm of hepatic cells. The results for ectromelia virus in mice differ in some ways from those obtained when hepatic cells are infected naturally from neighboring hepatic cells or macrophages (107). In the first place, large amounts of virus are needed for infection via the bile duct, and, secondly, cells do not contain antigen until 10 to 18 hr, instead of after 5 hr as when infection spreads naturally from adjacent hepatic cells. In spite of these differences, the following conclusions can be drawn from the results of bile duct experiments. Influenza and myxoma virus in mice, and also ectromelia virus in rats, infect periportal hepatic cells after bile duct injection, whereas they are taken up and digested by macrophages if they reach the liver via the blood stream. Hepatic cells infected in this way, however, are probably not capable of infecting neighboring hepatic cells, except in the case of ectromelia virus in mice. The experiments also showed that myxoma virus, for instance, infects mouse hepatic cells as readily as does ectromelia virus, when injected by the bile duct; rat hepatic cells are infected by ectromelia as readily as mouse hepatic cells. It is concluded that viruses sometimes fail to grow in hepatic cells because they have no opportunity to do so; they are taken up and inactivated by the macrophages in the sinusoids. Thus, although ectromelia infects the upper respiratory tract of rats (29) and myxoma virus infects suckling mice when injected intracerebrally (3), neither of these viruses grow in the liver after intravenous injection because they are destroyed by liver macrophages, which conceal the inherent ability of hepatic cells to support virus growth. Liver macrophages, considered in this way, protect hepatic cells from circulating viruses. Probably most nonpathogenic viruses are in any case incapable of growing in hepatic cells. This would clearly be true of tobacco mosaic virus (TMV), for instance, in the mouse.

Finally, some experiments will be described which show that viruses which normally infect macrophages and grow in liver cells may nevertheless be taken up, digested, and destroyed if they are premixed with specific immune serum. When ectromelia virus is mixed with vaccinia immune rabbit serum and injected intravenously into mice, the virus-antibody complexes, as stained with fluorescein-labeled antirabbit gamma-globulin antibody, are taken up by liver macrophages, and there is no reappearance of ectromelia antigen in macrophages or hepatic cells (Mims, *unpublished data*). The normally infectious virus is perhaps rendered digestible by the specific antiserum. In mice hyperimmune to ectromelia, the possible role of specific cellular immunity, as opposed to humoral immunity, is difficult to evaluate. When such mice are given massive intravenous injections of ectromelia, antigen, which can still be located in immune mice by the fluorescent-antibody technique, is taken up by liver macrophages as usual. Antigen then fades, as in normal mice, but nearly always fails to reappear (Mims, *unpublished data*). Very occasional macrophages can be seen to support virus growth and infect neighboring hepatic cells so that one or two small foci are present by 48 hr. These foci fail to expand further, their fluorescent outline becomes indistinct, and by 72 hr they no longer contain detectable antigen. In this way, a degree of immunity which protects mice from the intravenous injection of 10^9 lethal doses of ectromelia virus can be shown to be slightly less perfect at the histological level. Massive doses, however, are needed, doses which might occasionally and temporarily exhaust the supplies of specific antibody, so that a few small foci are initiated. It may be noted that a similar breakdown at the histological level in the natural resistance of rats to ectromelia can also be demonstrated by the fluorescent-antibody technique. When massive doses of ectromelia ($10^{9.0}$ pfu) are injected intravenously into rats, occasional hepatic cells are infected by 24 hr.

Uptake and passive transfer through macrophages to hepatic cells. Virus particles can, under some circumstances, pass across the macrophage lining of sinusoids and reach hepatic cells without a preliminary cycle of growth in macrophages. The first example is that of the intravenous toxicity of influenza virus in mice.

When a toxic dose of influenza virus is injected intravenously into mice and is taken up

by liver macrophages, infected hepatic cells begin to appear by 7 hr, so there could not have been a preceding cycle of virus growth in macrophages (110). Liver macrophages destroy a subtoxic intravenous dose of virus, but they are overwhelmed by the slightly larger toxic dose, and somehow allow virus to reach the susceptible hepatic cells. This occurs on a large scale, so that, in spite of the fact that the virus produced is largely noninfectious, most hepatic cells are infected *ab initio* and mice die with extensive liver damage. In this example, massive doses of virus are needed to overcome the macrophage barrier, and the hepatic cells could have been infected either by virus which had been phagocytosed and then egested into the space of Disse, or by virus which had "leaked" through submicroscopic gaps in the macrophage lining of sinusoids.

A breakdown in the macrophage lining of sinusoids is also sometimes seen when large doses of inert particles are injected intravenously. Parks and Chiquoine (121) showed that colloidal gold particles were present in the space of Disse only a few minutes after intravenous injection, and Hampton (71) showed the same for mercuric sulfide and thorotrast. A number of other inert particles have been shown to reach hepatic cells rather slowly after intravenous injection. Intravenously injected India ink, for instance, stays in mouse liver macrophages for many hours, but small amounts are present in hepatic cells after 3 days (Mims, *unpublished data*). Intravenously injected thorotrast (50), saccharated iron oxide (122), and beryllium (33) can also reach hepatic cells in small amounts after a few days.

The second example of a virus that is thought to pass through macrophages without necessarily infecting them, is (RVF) virus. RVF virus is almost certainly taken up from the blood of mice by liver macrophages and passed through to hepatic cells. Intravenously injected virus is cleared from the blood, and hepatic cells show typical nuclear changes as early as 1 hr later if large amounts of virus are injected (105). Inclusion bodies are seen in hepatic cell nuclei by 3 hr, and extensive hepatic cell damage then occurs. Mice die within 6 hr after such injections, and a combined study of virus production and histological changes indicate that there has been a single cycle of virus growth in nearly all hepatic cells. Although no fluorescent-antibody studies have been made, it seems probable that RVF virus is cleared from the blood by liver macrophages and infects hepatic cells very shortly afterwards. Growth in macrophages, if it occurs, is not a necessary part of the process, and most of the RVF virus particles which are taken up are passed straight through to hepatic cells. The above experiments were done with very large intravenous doses of RVF virus, but macrophages probably behave in the same way after small doses. They do not have to be flooded with virus before letting it through to hepatic cells, as in the case of influenza virus; RVF virus particles are treated in a fundamentally different way.

Presumably, one of the natural functions of liver macrophages is selectively to pass materials from the blood to hepatic cells. While this is normally done on a molecular scale, the evidence referred to above shows that it occurs under some circumstances with particulate materials, both viral and nonviral. Only two examples could be given of its occurrence with viruses, because in both cases intact virus reached hepatic cells and infected them, with pathological consequences. Virus particles which are readily digested by macrophages would in any case be unlikely to reach hepatic cells in the infectious state, although the products of digestion could do so. This would be the case with many nonpathogenic viruses, and with influenza virus in subtoxic doses. Sometimes the materials taken up from the blood by macrophages are excreted into the bile. This is so of certain metabolic products and drugs, and has also been shown to be true for certain inert particles. Juhlin (84) found that fluorescent methyl methacrylate particles appeared in the bile of rabbits within minutes after intravenous injection, as long as the particles were more than 0.1 μ in diameter. Intravenously injected thorotrast (50), manganese dioxide particles (47), and micrococcin particles (99) have also been shown to be excreted in the bile. Virus particles, too, can be cleared from the blood and excreted intact into the bile. When type I poliovirus is injected intravenously into cynomolgus monkeys (Howes and Mims, *unpublished data*), virus is recoverable in the hepatic bile within 1 hr. Large amounts of T_7 bacteriophage are present in the gall bladder bile of mice 2 hr after an intravenous injection (Mims, *unpublished data*). Perhaps other viruses can be excreted in

the same way, although their infectivity would not always be retained in the presence of bile. Particles could enter bile capillaries either from the space of Disse by passing through or between hepatic cells, or perhaps via the occasional Kupffer cell projections anchored directly to the bile capillaries (120).

In the light of the above discussion about the uptake of particles by liver macrophages and their subsequent transfer through to hepatic cells and bile, some observations can be made about the pathogenesis of infectious hepatitis virus infection in man. If the pathological changes in the liver are due to the growth of virus in hepatic cells—and this is by no means established (5)—macrophages must play an important part in the process. Virus would have to reach the liver from the blood, and would be taken up by the macrophages lining the sinusoids. These macrophages then, instead of digesting phagocytosed virus as they might digest a nonpathogenic virus, allow it to reach susceptible hepatic cells. It may be worth noting also that, if infectious hepatitis virus in the liver is excreted into the bile as is poliovirus in monkeys, there need be no other source for fecal virus in this disease. In homologous serum jaundice, virus might be absent from feces if it were inactivated by bile.

Uptake and growth in macrophages leading to infection of hepatic cells. This type of virus-macrophage interaction has been suggested for distemper virus in dogs (35), yellow fever virus in monkeys (154), and also more fully described for ectromelia virus in mice (107). In the ectromelia experiments, intravenously injected virus was very rapidly cleared by mouse liver macrophages, so that after 5 min more than 90% of the inoculum was present in the liver. Fluorescent-antibody studies showed that antigen in liver macrophages had faded considerably within 0.5 hr, but this was the fading of antigen that accompanies an eclipse period, because 7 hr later there was a rise in liver titer together with the reappearance of antigen in some macrophages. After the growth of virus in these macrophages, numbers of hepatic cells were infected which, about 5 hr later, were seen to contain antigen and produced a further rise in liver titers. These in turn infected adjacent hepatic cells, and so on until, after about five cycles of hepatic cell growth, there were very few uninfected cells and mice died shortly afterwards. Growth in macrophages, as judged by fluorescent-antibody studies, seemed to be an essential preliminary to hepatic cell infection, however much virus was injected. A more limited ability to grow in macrophages and then infect hepatic cells was shown by rabbitpox virus. Macrophages were infected much less readily than with ectromelia virus, and, even when very large amounts of virus were injected intravenously, only a small number of foci were established in the liver. Once hepatic cells were infected, however, the foci enlarged, and, although in some mice foci failed to appear at all, in others there were enough to produce by their enlargement extensive liver damage.

There is evidence that not all liver macrophages are infected with equal readiness by ectromelia virus. It was observed (107) that only about one in four of the macrophages which took up virus were in fact infected, even when massive doses were given. Nevertheless, it is difficult to be sure that this was due to genuine differences in infectibility, because macrophages are likely to take up different amounts of virus. Rapidly cleared particles may have been largely removed from the sinusoidal blood by the time it reaches the central vein, so that macrophages in this region tend to take up fewer particles. Apart from their position, individual macrophages at any given time probably differ in phagocytic activity, and Parks (119) showed that different liver macrophages take up different amounts of injected particles, whatever their position in the lobule. If, then, macrophages take up different amounts of ectromelia virus, those with smaller amounts would tend to yield virus much later (107), perhaps not until their neighboring hepatic cells had been infected by the enlargement of already established hepatic cell foci. These late-yielding macrophages, appearing in established foci, would be very difficult to detect by the fluorescent-antibody technique.

The experiments with intravenously injected ectromelia virus also give some information about the way in which macrophages infect hepatic cells. Only a small proportion of liver macrophages yielded virus, however much was injected, and, if virus then spread easily along the space of Disse, one infected macrophage could infect a large number of hepatic cells. Most hepatic cells could be infected from the start, in spite of the small proportion of infected macrophages. In fact, the hepatic cells infected

in the first cycle of growth were quite sparsely scattered in sections, and this suggests that macrophages only infected the immediately adjacent hepatic cells. Careful histological studies have shown that in the mouse liver there are about three hepatic cells for every two littoral cells (51), so that macrophages are probably in contact with only a small number of hepatic cells. In those instances where nearly all hepatic cells are infected *ab initio*, as after massive intravenous injections of influenza or RVF virus (see above), there has doubtless been a widespread passage of virus through the macrophage lining of sinusoids. If a massive injection of ectromelia virus could produce a similarly widespread infection of liver macrophages, all hepatic cells would be involved in the next growth cycle, instead of after at least four more cycles of growth, as was in fact observed.

In rabbits injected intravenously with rabbitpox virus, the liver macrophages, and thus hepatic cells, are infected at an early stage of the disease (Mims, *unpublished data*), just as they are in mice with ectromelia virus. Myxoma virus, on the contrary, behaves differently in the rabbit liver. From the fluorescent-antibody studies now to be described, further conclusions can be reached about the part liver macrophages play in virus infections. When rabbits are infected intradermally with the virulent standard laboratory strain of myxoma virus (57), infected hepatic cells are seen with the fluorescent-antibody technique at the time of death (Fig. 2). Earlier in the disease, there are occasional antigen-containing cells in the liver sinusoids, but the spread to hepatic cells is a late phenomenon. There are no infected hepatic cells a few days before death and, even at the time of death, the infected hepatic cell foci are still small, although there is a very marked periportal accumulation of mononuclear cells, many of which contain antigen. In seven rabbit livers examined at the time of sickness or death, all except one contained scattered infected hepatic cells. Never more than 10% of hepatic cells were involved, except in one rabbit where nearly 50% were infected. The foci were nearly all small, consisting, in sections, of fewer than ten hepatic cells. The late appearance of hepatic cell foci, which never have time to become very large, could be attributed to a terminal breakdown in the protection of hepatic cells by liver macrophages. Viremia is detectable

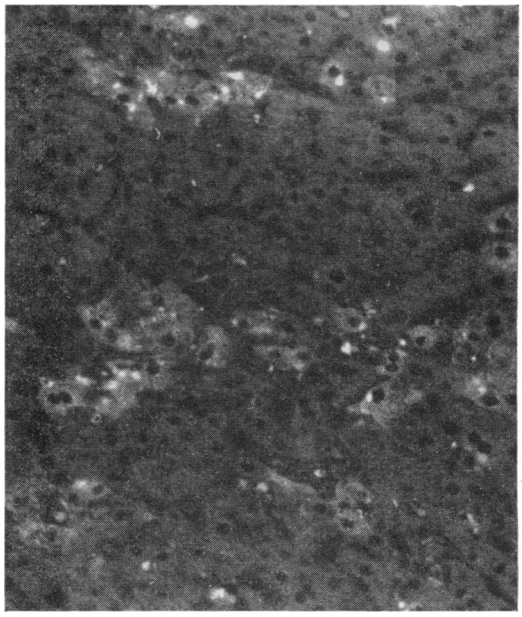

FIG. 2. *Fluorescent-antibody stained section of liver from rabbit dead 9 days after intradermal injection of standard laboratory strain of myxoma virus. Moderate numbers of infected hepatic cells.* ×*400.*

within 2 to 3 days of infection (56), and circulating virus, free or in the form of infected cell debris, tends to be removed by liver macrophages. Nevertheless, macrophages are not infected until late in the disease, and virus does not until then have an opportunity to infect hepatic cells. That a terminal breakdown occurs in the protection of hepatic cells by macrophages is supported by the following observations. Rabbits were injected intravenously with more than 10^8 ID_{50} of the SL strain of virus, a procedure which is assumed to deposit virus in liver macrophages. In spite of this, no hepatic cells had been infected by 4 days, and only small foci of infected hepatic cells were present in a rabbit dying on the seventh day.

The evidence discussed in this section shows that different viruses differ markedly in their ability to grow in liver macrophages and thus infect hepatic cells. Virulent strains of ectromelia infect liver macrophages more readily than do other viruses, but even here only about one in four macrophages are infected. They are infected even less readily by avirulent strains of ectromelia virus (see following section), and by

certain strains of vaccinia virus. The liver macrophages of rabbits cannot be infected at all by myxoma virus until late in the disease.

Macrophages and Susceptibility to Virus Infections

Macrophages are literally in a position to control the susceptibility of animals to virus infections, since they monitor the main body compartments and may control the entry of viruses to target organs like the liver.

Some of the findings discussed above show how the susceptibility of animals to viruses may be determined at the macrophage level. For instance, it was shown above that ectromelia virus is destroyed in the macrophages lining the liver sinusoids of rats and thus has no opportunity to express its intrinsic ability to grow in hepatic cells. Evidence was presented that myxoma virus, too, fails to grow in the liver of mice for the same reason.

The importance of macrophages in susceptibility to virus infections was demonstrated by Bang and Warwick (8), who showed that the genetic difference in susceptibility of two strains of mice to mouse hepatitis probably operates at the macrophage level. Susceptible mouse liver macrophages degenerated when infected in vitro, whereas macrophages from resistant mice survived. Similar findings were reported for lung and heart macrophages. Theis and Koprowski (152) produced evidence that the susceptibility of mice to West Nile virus also has a basis in the macrophage response. Little or no virus was produced by splenic and peritoneal macrophages from resistant strains of mice after infection in vitro, whereas large amounts of virus were produced by susceptible mouse macrophages.

More conclusive evidence for the role of macrophages has now been presented by Roberts (128). Roberts used the fluorescent-antibody technique to analyze the differences between the avirulent Hampstead Egg and the virulent Hampstead Mouse strain of ectromelia virus, and produced strong evidence that the Hampstead Egg strain was avirulent because of a lessened ability to infect mouse macrophages. After intravenous injection, the avirulent Hampstead Egg strain of virus infected liver macrophages with much greater difficulty, and macrophages yielded fewer infective particles than in the case of the Hampstead Mouse strain. This difference in macrophage response was confirmed when quantitative in vitro studies were made of the susceptibility of mouse peritoneal macrophages (Roberts, *in preparation*). Once growth in hepatic cells had been initiated, however, no differences between the strains could be detected. In an infectious process where the outcome depends on the race between growth in liver and lymphoid tissue on the one hand (see below), and the immune response on the other, a delayed initiation of growth in these target organs may be of crucial importance.

Action of Other Microorganisms on Macrophages and Thus on Virus Infections

Microorganisms which influence the function of macrophages could thereby theoretically change the susceptibility of animals to virus infection, but there is as yet little evidence that this ever occurs. If, for instance, blood virus clearance mechanisms were altered in malaria, this could conceivably affect viremia levels in arthropod-borne virus infections. Barnett (10) showed that malaria-infected canaries had lower Western equine encephalitis viremias, but the reason is not known. An interaction between two diseases like smallpox and malaria, both of which (27, 151) involve macrophages, might also be expected. Tuberculosis, known to have profound effects on macrophages (21), might also affect the course of certain virus diseases. Old et al. (115) made the suggestion that a number of agents, including BCG and endotoxin, which affect the response of mice to intravenously injected Mengo virus, do so by means of an action on the reticuloendothelial system.

The enhancing effect of *Eperythrozoa coccoides* on the pathogenicity of mouse hepatitis virus (113) is perhaps attributable to its action on macrophages. The histological reaction in the dual infection is characterized by the formation of multinucleated giant cells in liver veins (65), and presumably Kupffer cells also are involved. If mouse hepatitis virus needs to cross the liver macrophage barrier before infecting hepatic cells (see above), then this might be accomplished more readily if liver macrophages were infected with *E. coccoides*.

Finally, the endotoxins produced by gram-negative and other bacteria have a profound effect on macrophages (22, 153), and the influence of injected endotoxins on the course of virus infections could at least partly be explained in

this way. The influence of injected endotoxin on the resistance of mice to ectromelia virus was described by Gledhill (66). The endotoxin which is normally produced in the gut might also be important in virus diseases where macrophages are damaged, for the following reason. There is good evidence that, in rabbits at least, endotoxin is constantly being absorbed from the alimentary canal under normal circumstances, and then taken up and inactivated by macrophages (125). Intravenous injections of thorotrast, and hemorrhagic or traumatic shock, are probably lethal to rabbits because of their effect on macrophages. The uptake and inactivation of endotoxin by macrophages is impaired, and rabbits then die as a result of the action of endogenous endotoxin. The possibility that rabbits infected with myxoma virus die in the end because of a failure of macrophages to inactivate endogenous endotoxin was therefore investigated. As will be seen, no evidence for this possibility could be obtained. Death in myxomatosis is not attributable to the growth of virus in a target organ because, apart from the skin where there is extensive virus growth, fluorescent-antibody studies showed limited involvement of adrenals, kidneys, spleen, liver, lung, and brain (Mims, *unpublished data*). An alteration in macrophage function late in the disease was suggested above, and conceivably rabbits die as a result of the action of absorbed endogenous endotoxin. Inconclusive results, however, were obtained in experiments where rabbits infected intradermally were given small graded doses of endotoxin intravenously a few days before death was expected (Mims, *unpublished data*). There was, at the most, only a very slight increase in sensitivity to endotoxin. Endotoxin sensitivity, therefore, is not the cause of death in myxomatosis. Perhaps an understanding of such matters awaits advances in our knowledge of the role of polypeptides or other agents in the production of shock.

Rabbits are particularly sensitive to endotoxin; mice, for instance, are only $\frac{1}{500}$ as sensitive. Thus, although mice late in infection with ectromelia virus succumb to one-tenth the normal lethal intravenous dose of endotoxin (Mims, *unpublished data*), this dose is so large that it is unlikely to be absorbed from the gut to play an important part in the disease.

Use of Thorotrast in the Study of Macrophages in Virus Diseases

Thorotrast is one of the most effective of the macrophage-blocking agents. It is taken up by reticuloendothelial macrophages after intravenous injection, small doses impairing their function and larger doses having a more marked effect. After the intravenous injection of thorotrast to mice, carbon clearance rates are greatly reduced, and carbon has the opportunity to localize in capillary endothelium (16). The destructive action of intraperitoneally injected thorotrast on peritoneal macrophages is described in the section below. Thorotrast, therefore, might prove to be a useful experimental tool for the study of macrophages in virus diseases.

Thorotrast certainly reduces the clearance rates of intravenously injected viruses. Brunner et al. (28) showed that there were reduced NDV clearance rates in mice 5 hr after the intravenous injection of 0.25 ml of thorotrast, and the same was found to be true for vaccinia virus (Mims, *unpublished data*). With vaccinia virus, however, the depression of clearance was greater 18 hr after the injection of thorotrast, suggesting that the thorotrast effect may take some time to develop. This was confirmed by the observation that the greatest effect on Semliki Forest virus viremia was seen 12 to 18 hr after the injection of thorotrast (see below).

The action of thorotrast on the course of a virus infection was then investigated, by use of the fluorescent-antibody technique. Large doses of the virulent Moscow strain of ectromelia virus were injected intravenously to mice 18 hr after 0.3 ml of thorotrast. The results showed (i) that at least some macrophages loaded with thorotrast could nevertheless be infected and yield antigen. Thus, individual macrophages may be capable of taking up virus particles, albeit in small quantities, in spite of the fact that they contain thorotrast. (ii) Foci in the spleen enlarged much more rapidly in thorotrast-treated mice, suggesting that there had been some breakdown in the control of infection by macrophages (see below). (iii) In the liver of thorotrast-treated mice, hepatic cell foci appeared at a time when Kupffer cells only were infected in control animals, suggesting again that there had been some breakdown in the control of hepatic cell infection by macrophages. Hepatic cells were not infected by

10 hr, so there was no evidence that intravenously injected virus straightaway infected hepatic cells in thorotrast-treated mice. Thorotrast-treated mice had no more virus growth in either lung or kidney, both of which contained thorotrast, than did normal mice.

It is doubtful, however, what significance to attach to the above experiments with ectromelia virus. Thorotrast, in doses twice as large as the reticuloendothelial blocking dose, kills normal mice within 24 hr, undoubtedly producing widespread effects on bodily functions. Any action of the blockading dose on virus infections, it is felt, may not necessarily occur by way of the action on macrophages. In any case, the fact that macrophages loaded with thorotrast are nevertheless occasionally infected means that thorotrast is not a completely reliable macrophage-blockading agent in virus infections. The whole subject of reticuloendothelial blockade is in a confused state, and more studies of the kind recently reported by Murray (111) are clearly needed. Murray suggested that many blockading agents are effective because they share common surface properties with the test material. This is a different and more specific action from that assumed to occur with thorotrast as used above, which perhaps acts as a blockading agent only, insofar as it damages macrophages.

VIREMIA

In most virus infections, organs and tissues are involved which are distant from the site of initial entry of virus into the body, and the spread to these organs takes place via the blood stream. There is a viremic stage in the disease, whether detectable or not. This is as true for ectromelia in mice as it is for poliomyelitis in man. In the case of arthropod-borne virus infections, the importance of viremia is even more obvious, adequate viremia levels being essential for the spread of virus to fresh hosts. Nevertheless, very few studies have been made which throw any light on viremia mechanisms, and the principal considerations in understanding the viremic state have hardly been enunciated.

If viruses in the blood are normally removed for the most part by macrophages, then viremia must be considered in relation to the rate of removal. The types of viremia can be classified as follows.

Cell-Associated Viremia

White cells. In mice infected with ectromelia virus (107), virus in the blood is largely associated with the blood cells, particularly platelets, lymphocytes, and monocytes. Lymphocytes and monocytes can be seen to be infected when blood smears are stained with fluorescent antibody (Mims, *unpublished data*). Degenerating cells and cell fragments containing antigen are occasionally seen, but as cells become damaged or disrupted as a result of infection they are no doubt cleared, so that at any given time most of the blood virus is associated with circulating intact host cells. When such blood is injected into a normal mouse, it is poorly cleared, as compared with the clearance in 2 min of 95% of ordinary virus preparations after intravenous injection. In the viremia of ectromelia, therefore, virus is associated with the formed elements of the blood, and tends not to be cleared. Clearance mechanisms are intact until a late stage in the disease, because large intravenous injections of virus are cleared as in normal mice (107). The defective clearance seen late in the disease could be because of depressed activity on the part of liver macrophages, or perhaps because of the impaired circulation of blood through the liver thought to occur at this stage. A similar kind of viremia probably occurs in myxomatosis. Fenner and Woodroofe (56) showed that, in rabbits infected with myxomatosis, virus in the blood was associated with white blood cells, probably lymphocytes. In the viremias of fowl plague (155), ephemeral fever of cattle (93), rinderpest (41), distemper (131), and measles (117), virus has been shown to be associated with leukocytes.

One important consequence of a white cell-associated viremia is that leukocytes can then carry virus with them in their migrations through the body. The dissemination of infection in this way throughout the lymphatic system will be discussed below. Lymphocytes and monocytes are more important than polymorphs (see below), and a knowledge of the life and movements of these cells will help in understanding the part they play in virus diseases.

Red cells. In some viremias, blood virus is associated with red cells. This is usually because it is adsorbed to red cells rather than because it is present inside them, or has grown in them.

Adsorption to erythrocytes is demonstrable in vitro, and is the basis for hemagglutination tests. Viruses carried on red cells would circulate for longer than free virus, even if the red cells had a shorter circulatory half-life than normally; this might have an important influence on the initiation and maintenance of viremia. This kind of viremia can be compared with the parasitemia of malaria where parasites circulate inside red cells, free forms being removed from the plasma by macrophages or by entering uninfected red cells.

The first example of a red cell-associated viremia is that seen in certain arthropod-borne virus infections. In RVF virus infections, for instance (101), virus is present in the red-cell fraction of the blood, and cannot be removed by repeated washing. Probably free virus liberated into the plasma is secondarily adsorbed to red cells. The red cell-associated virus forms a small proportion of the total blood virus, however, and it is unlikely that it contributes significantly to the viremia mechanism.

The second example is the transient viremia demonstrable in mice infected with influenza virus by aerosol (72), where virus was shown to be associated with the red-cell fraction. Influenza virus elutes from erythrocytes, but this in itself would not limit the time for which it could be carried in this form in the blood, because of the very large number of erythrocytes available. It is perhaps best, however, to regard the attachment of influenza virus to erythrocytes as an irrelevant consequence of its ability to combine with receptors, rather than a property which is of any importance in the infected animal. For a virus which is inhaled and infects respiratory epithelium, viremia is in any case of no consequence except for the transport of virus antigen to antibody-producing organs like the spleen, and white cells could do this more readily than red cells.

The third example is the viremia of hog cholera. Very high blood titers occur, and most of the virus is red cell-associated (123). The disease is not transmitted by arthropods, however, and viremia can be regarded as incidental to the spread of virus to tissues and secretions. The fact that it is red-cell associated is unlikely to be of great importance.

The last example is the viremia seen in mice congenitally infected with lymphocytic choriomeningitis (LCM) virus, which is partly red cell and partly plasma in type. Virus cannot be washed from red cells (157; Mims, *unpublished data*), and is adsorbed by mouse red cells in vitro (Mims, *unpublished data*). In most virus infections, the time for which a red-cell viremia could operate would be limited because of antibody production, but in infections of this sort, where antibody is not produced, virus could circulate for as long as the red cells to which it is attached. The normal circulatory half-life of mouse red cells is about 20 days (145). A red-cell viremia could readily be established in this infection if virus grew in the bone marrow and became attached to newly formed red cells. It would be of little importance however, if, as is suggested below, LCM virus is in any case not cleared from the blood of congenitally infected mice.

Plasma Viremia

Clearance rate normal. In such cases, the clearance of virus from the plasma proceeds at the normal rate. If this clearance rate is rapid and the entry of virus into the plasma is comparatively slow, there may be no detectable viremia. This presumably could occur in any virus disease where there is generalization in the absence of detectable viremia. If the rate of entry of virus is equal to the rate of removal, the viremia will be steady, whereas an increase or decrease in the rate of entry of virus would be accompanied by a rising or falling viremia.

The following experiments with Semliki Forest virus indicate that viremias in some arthropod-virus infections are of this type. It was at first thought possible that, during viremia, plasma virus was in a form that could not be cleared. Perhaps virus particles were coated with plasma proteins and therefore not regarded as foreign. When Semliki Forest virus viremia blood was injected intravenously into an uninfected mouse, it was cleared, but donor mouse plasma proteins could have been regarded as foreign by the recipient, which therefore cleared the particles. This possibility was excluded when it was found that whole viremic blood from one mouse of the genetically homogeneous Bagg strain was cleared in a litter mate just as if the donor had been from a different strain of mouse. In Semliki Forest virus viremia, therefore, plasma virus is not in some particular state which prevents its clearance. The effect on viremia of an artificial reduction of

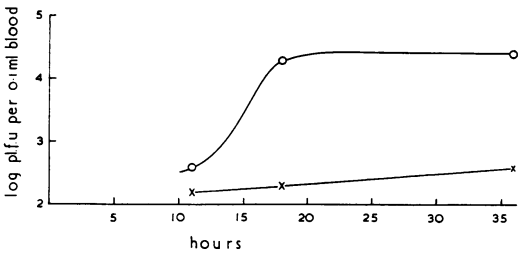

FIG. 3. *Effect of thorotrast on Semliki Forest virus viremia. Four adult mice were infected subcutaneously with Semliki Forest virus and at the same time 0.3 ml of thorotrast was given intravenously to two of these mice. Pooled tail blood titers obtained by plaque assay on chick embryo fibroblasts from the pair of thorotrast-treated* (O) *and the pair of control* (X) *mice.*

the clearance rate was then investigated. Thorotrast-treated mice have a reduced capacity to clear intravenously injected Semliki Forest virus (Mims, *unpublished data*), and mice were infected subcutaneously with virus, treated with thorotrast, and blood titers were followed. There were very significant increases in viremia levels as compared with control mice (Fig. 3). These experiments suggest that Semliki Forest virus is being continuously shed into the blood and at the same time cleared by reticuloendothelial macrophages. Thorotrast upsets the balance by reducing the rate of clearance, and as a result there is an increase in the blood virus level.

The viremia in monkeys infected with poliovirus is of the plasma type, and here, too, clearance is thought to be normal. Cynomolgus monkeys develop a viremia 1 day after large doses of the Mahoney strain of virus are painted into the tonsils, but on the second day they still clear intravenously injected virus in the usual way (Howes and Mims, *unpublished data*).

Clearance impaired. Although theoretically there could be an impaired clearance rate in any virus disease, in most cases there is no good evidence that it occurs or contributes significantly to the viremia. The clearance of any given test particle may not be relevant because virus may be cleared by specific mechanisms, perhaps depending on adequate blood levels of specific opsonins. It is nevertheless difficult to avoid the conclusion that some plasma viremias are maintained by an impairment of clearance, and that this is a failure to clear a particular type of virus rather than a general depression of clearance. The prolonged viremias seen in homologous serum jaundice infection (149), in swine infected with hog cholera virus (7), and in mice infected with LCM virus (157) are probably of this type. It seems unlikely that there is a continued removal of plasma virus by reticuloendothelial macrophages, because this would involve improbable chronic infection rates in tissues lining blood vessels or lymphatics. On the other hand, a general depression of reticuloendothelial cell function is also unlikely. If, in these infections, plasma virus is not cleared at the expected rate, the most satisfactory explanation is that it is due to immune tolerance. Circulating antibodies are not detectable in many of these infections, which begin in utero or in the early neonatal period, and the failure to recognize virus particles as antigenically foreign is perhaps accompanied by a failure of macrophages to remove circulating virus. Unfortunately, there is no experimental evidence on this matter. Mice congenitally infected with LCM virus have a chronic viremia, and when they are injected intravenously with a guinea pig spleen preparation of the same LCM strain, the injected virus is cleared down to the level of the prevailing viremia (Mims, *unpublished data*). Such an experiment, however, cannot be considered significant because of the large amounts of antigenically foreign guinea pig spleen material which would inevitably contaminate such an unpurified virus preparation.

Thus, it seems likely that in the chronic viremias mentioned above, infected animals do not recognize plasma virus as foreign and therefore fail to remove it from the blood. Even so, there would have to be a slow rate of entry of virus into the blood to replace thermally inactivated virus. The in vitro half-life of LCM virus as it occurs in the plasma of congenitally infected mice was found to be less than 24 hr. Even if no infectious virus was removed, thermally inactivated virus, if antigenically distinct as in the case of poliovirus (26, 132) might, of course, be cleared.

LOCALIZATION OF VIRUSES IN CAPILLARY ENDOTHELIUM

There are good reasons for supposing that virus particles are able to localize and grow in the endothelium of capillaries or larger blood vessels. If they can do this, two important features in the pathogenesis of virus infections are accounted

for. First, virus which has grown in vascular endothelium could be the source of virus in plasma viremias (see above) and, second, the way in which viruses in the plasma invade certain target organs could be by growing across capillary endothelial barriers.

A growth across capillary endothelium must certainly be considered when viruses invade organs whose vascular bed consists of capillaries. It has often been suggested for viruses invading central nervous tissue, and would also explain the invasion of organs like the pancreas or testicle. In poliomyelitis and the arthropod-borne encephalitides, the passage of virus across the blood-brain barrier accounts for many features of the disease as well as most experimental findings (80, 86, 104). Barton et al. (12) described inclusion bodies in vascular endothelium in the brain of foxes infected with fox encephalitis virus, but unfortunately there is no direct evidence available for the above diseases, evidence which could be provided by the fluorescent-antibody technique. Virus, of course, might pass across capillary endothelium without infecting the capillary endothelial cells themselves, but a primary infection of pericapillary glial cells should then be demonstrable. There is direct fluorescent-antibody evidence that distemper virus in dogs (35) and the NWS strain of influenza virus in chick embryos (78) grow in capillary endothelium, and this is the type of evidence that is needed for the enteroviruses and arboviruses. If viruses cross the blood-brain barrier by growing across capillary endothelium, it is, of course, possible that capillary endothelium in particular areas of the central nervous system are involved. There may even be a preliminary passage of virus across the capillaries of the blood-cerebrospinal fluid barrier before invasion of nervous tissue.

When viruses invade organs whose vascular bed consists of sinusoids, the situation is quite different because these sinusoids are lined by macrophages. Also, a growth through capillary endothelium need not be considered when viruses in the blood are associated with leukocytes and could be carried across capillary barriers by diapedesis. Platelets, if they carry virus, might in the course of their normal activity attach to capillary endothelium, but they would infect rather than pass through this cell barrier. A passage of infected leukocytes across capillary endothelium is a possible way in which pox-viruses initiate the skin lesions of the generalized rash. This does not exclude the infection of capillary endothelial cells, and Tyzzer (158) described inclusion bodies in endothelial cells lining small blood vessels in the skin lesions of human cases of varicella. It should be noted that much of the experimental work on the localization of viruses in the skin is difficult to interpret. Ectromelia virus, for instance, localizes and grows in plucked or scarified skin areas after intravenous injection (Mims, *unpublished data*), and similar findings were reported by Whiteley (163) for rabbits infected with vaccinia virus. In the case of ectromelia virus, however, no such localization could be demonstrated during the secondary viremia following natural routes of infection (Roberts, *unpublished data*). It is thought that although intravenously injected particles can localize in "sticky" or inflamed capillary beds, the white cell-associated virus in the secondary viremia does not necessarily behave in the same way.

Virus which has grown in the endothelium of capillaries or larger blood vessels could be the principal source of blood virus. In hog cholera virus infections, for instance, where pathological changes and inclusion bodies are common in the endothelial cells lining small blood vessels (140), blood virus could well come from this source. In arthropod-borne virus infections, however, viremia levels are of more fundamental importance, because transmission can only take place if enough virus is present in the blood. Such viruses are to some extent introduced directly into the blood by arthropod vectors (69), and the source of blood virus must be cells with access to the blood. These could be either the cells in the blood, the lymph entering the blood, or the cells lining blood vessels. If these viremias were maintained by virus yielded from infected blood cells, presumably leukocytes, one would expect hematological changes to be more profound than they are, especially in infections with high-level viremias. The lymph, too, is thought to be an inadequate source of blood virus, at least in some cases. Malkova (95, 96, 97) showed that tick-borne encephalitis virus enters the blood from lymphatics, but it is difficult to imagine that the lymph could be the principal source of virus in suckling mice injected intracerebrally, and thus intravenously (31) with Semliki Forest virus. These mice produce peak viremias (10^{10}

pfu/ml of blood) within less than 24 hr. The source of such very large amounts of virus, it is thought, must be the cells lining blood vessels. If it were the cells lining blood vessels, this would include reticuloendothelial as well as capillary endothelial cells. In experiments where the organs of adult and suckling mice in the early stages of Semliki Forest virus viremia were sectioned and stained with fluorescent antibody, there was no indication that reticuloendothelial cells in the liver, spleen, or adrenals were involved (Mims, *unpublished data*). The growth of such viruses in capillary endothelium has still to be directly demonstrated.

The capillary endothelium in certain parts of the body may be involved in virus diseases. This could be due, first, to random localization of virus in capillary endothelium throughout the body, but growth only in certain regions. On the other hand, virus might localize only in capillary endothelium in certain parts of the body, either because of local inflammatory conditions or because the capillary endothelium differs in some other way and has a greater affinity for virus particles. The possibility that capillary endothelium differs in different organs is not a new one. Pressman et al. (124) produced evidence that the specific antigenicity of organ extracts may reside in the vascular bed of the organ, and it has been observed that fluorescein-coupled antibody to mouse brain specifically stains the vascular bed of this organ (Mims, *unpublished data*).

If viruses do in fact grow in capillary endothelium, some predictions can be made about the circumstances under which this is possible. When large particles are injected intravenously, they do not normally stick to and enter capillary endothelial cells very readily, and reticuloendothelial clearance is rapid. It can be said with some confidence that viruses cleared from the blood as readily as ectromelia would, as free particles, have little or no opportunity to reach capillary endothelial cells. Smaller viruses like Semliki Forest virus, however, which have an intravascular half-life of 20 to 30 rather than 2 min, would have more opportunity to infect capillary endothelium because they stay longer in the blood. This would occur more readily if virus particles had some particular affinity for capillary endothelial cells. Clearance curves for such viruses could in fact represent localization in capillary endothelium as well as in reticuloendothelial cells. Reticuloendothelial clearance, however, might still limit the extent to which virus localized in capillaries, and this thought prompted the following experiment. When Semliki Forest virus is titrated in adult mice by different routes of injection, intravenous lethal end points are one-hundredth as high as subcutaneous end points (Mims, *unpublished data*). This perhaps means that only a small proportion of the injected virus localizes in the brain, and an attempt was made to promote capillary localization by blocking reticuloendothelial cells with thorotrast. Benacerraf et al. (16) showed that intravenously injected carbon localized in capillary endothelium when reticuloendothelial clearance was impaired, and this could be achieved by giving repeated intravenous doses of carbon or blockading injections of thorotrast. The Semliki Forest virus intravenous end point, however, was unaffected whether mice received intravenous thorotrast 5 or 18 hr before the titration. Thorotrast is known to impair the clearance of Semliki Forest virus from the blood, and there are two possible conclusions from the experiment. Either the localization of Semliki Forest virus in capillary endothelium is not much affected by reticuloendothelial clearance or, more probably, subcutaneous titers are higher because virus grows in certain cells before entering the blood, which increases the effective dose entering the blood.

Finally, it is necessary to discuss rickettsial diseases, because the growth of rickettsiae in capillary endothelium has been established histologically (166). It seems likely, although there is no experimental evidence, that rickettsiae in the plasma are normally rapidly removed by the macrophages lining sinusoids. [Rickettsiae also occur in circulating leukocytes (44, 45, 112, 114).] If this is so, the following suggestions about the course of rickettsial infections can be made. Unless rickettsiae have some particular affinity for capillary endothelial cells, they can only localize in them with any frequency if clearance by macrophages is impaired. Rickettsiae entering the blood during the incubation period of rickettsial diseases would be removed by reticuloendothelial macrophages, but if, late in the incubation period, clearance by macrophages was impaired, there would then be the large-scale infection of capillary endothelial cells which produces clinical disease. There is no doubt that macrophages are intimately involved in rickettsial

diseases; an extensive macrophage reaction is seen in organs like the liver as well as in the blood vessel lesions (165).

The toxic action of rickettsiae can also be explained if it is assumed that rickettsiae do not often localize in capillary endothelium unless clearance by macrophages is impaired. The physiopathology of rickettsial toxicity in rats and mice has been carefully analyzed, and it is now clear that the capillary damage, extravasation of fluid, hemoconcentration, and vascular collapse are attributable to an early and widespread interaction of rickettsiae with capillary endothelial cells (161). Enormous doses of rickettsiae are needed. Rickettsiae given in small doses probably localize in macrophages, and hence mice injected intravenously with small doses of murine typhus rickettsiae (118) or infected with Q fever (30) develop focal liver lesions as a sequel to the involvement of liver macrophages. Moderate-sized intravenous doses of rickettsiae are probably dealt with in the same way, but, when the massive toxic dose is injected, clearance by macrophages is perhaps temporarily inadequate. Rickettsiae then have the opportunity to localize in capillary endothelium, and the toxic action is seen. If this is in fact so, one would expect the toxicity titer of a rickettsial preparation to be substantially higher in mice with reduced ability to clear circulating rickettsiae.

In summary, many viruses probably grow in capillary endothelium or the endothelium of larger blood vessels, although this has not often been directly demonstrated by the fluorescent-antibody technique. The growth of virus in capillary endothelium explains how viruses in the blood invade organs like the brain, and virus produced in vascular endothelium could well be the main source of blood virus in arthropod-borne virus infections. If all viruses in the blood are cleared by reticuloendothelial macrophages, those cleared very rapidly would probably have little opportunity to localize in capillary endothelium. Thus, rickettsiae, if they are cleared very rapidly, could not often localize in capillary endothelium unless clearance by macrophages was impaired. Small viruses, with longer circulatory half-lives, would have more opportunity to localize in capillary endothelium, particularly if it was inflamed or had some particular affinity for virus particles.

LYMPHOID TISSUE IN VIRUS INFECTIONS

Much has been written about the possible role of lymphoid cells and the lymphatic system in virus diseases, but speculations have often had to take the place of firm conclusions because of the very limited experimental evidence available. Lymphocytes are ubiquitous cells. They are produced in vast numbers, particularly in immature animals that are experiencing their first encounters with infectious agents, and they circulate repeatedly through the body, carrying out functions which are only just beginning to be revealed. The lymphatic circulatory system drains nearly all tissues and is known to be important in the disposal of injected particles; it is not difficult to imagine an important role for this system in virus infections. It is in lymphoid tissue, moreover, that the immune response occurs, and the plasma cells which produce antibody are located in this tissue. Here, too, macrophages occur in intimate relation with lymphoid cells, and the importance of macrophages in virus diseases was pointed out above. Lymphoid tissue, in short, is obviously likely to play an important part in virus infections. Unfortunately, it has been inadequately studied, even by the routine titration approach (see Introduction), and nothing is known at the cellular level of the course of infection in lymphoid tissue. In many virus infections, of course, there are histological changes in lymphoid tissue, but there is no information as to the relative roles of adrenal cortical hormones and the immune response, as opposed to the infection of cells, in causing these changes. For instance, are small lymphocytes ever themselves infected? This is clearly a great opportunity for the fluorescent-antibody technique, which can show what cells are infected, and trace the spread of infection through the lymphatic system histologically.

In the section which follows, unpublished observations on the lymphatic system and lymphoid tissue in mice infected with ectromelia virus are described and discussed. This is followed by a short discussion of the immune response in virus infections, with special reference to macrophages. Although nearly all the experimental observations are about ectromelia, which may be an unusual virus in its ability to grow in and destroy lymphoid tissue and macrophages, it is thought that many of the general conclusions can probably be applied to other virus infections.

Spleen

Although the spleen is not a vital organ like the liver, and can be removed with impunity in normal mice, it is important in virus infections for three reasons: first, because it is an antibody-producing organ; second, because splenic macrophages, like liver macrophages, monitor blood and take up circulating virus particles; and, third, because circulating lymphoid cells and macrophages tend to localize in the spleen, and these cells could themselves carry virus or be infected.

The spleen is an important antibody-producing organ, particularly when viruses are injected intravenously. Thus, if mice whose resistance to ectromelia is attributable to a superior immune response are splenectomized, they then become susceptible to intravenously injected virus (138). On reaching the spleen, circulating virus particles are treated like inert particles, being taken up by the macrophages lining splenic sinusoids. Even if particles pass into the extravascular regions of the red pulp, they will probably be taken up by macrophages, and there is evidence (162) that these regions consist in part of collapsed sinusoids. This phagocytosis is then an inevitable first step in the immune response, and, if virus particles fail to grow in or damage macrophages, they suffer the fate of other non-replicating antigens. The subsequent events in the spleen constitute the subject of immunology itself, and no further mention of them will be made here. Alternatively, virus particles reaching the spleen in this way might grow and produce tissue damage, so that there is a struggle in the spleen between the immune response and the destructive activity of the virus. Finally, virus particles might reach the spleen not free in the blood, but on or in circulating lymphoid cells or monocytes. The lymphoid cells, as long as they behaved normally, could move into the lymphoid follicles and initiate infection independently of macrophages. Infection would not proceed independently of macrophages, because macrophages feature prominently in the white pulp of the mouse spleen (62).

Fluorescent-antibody studies on the behavior of ectromelia virus in the spleen of mice will now

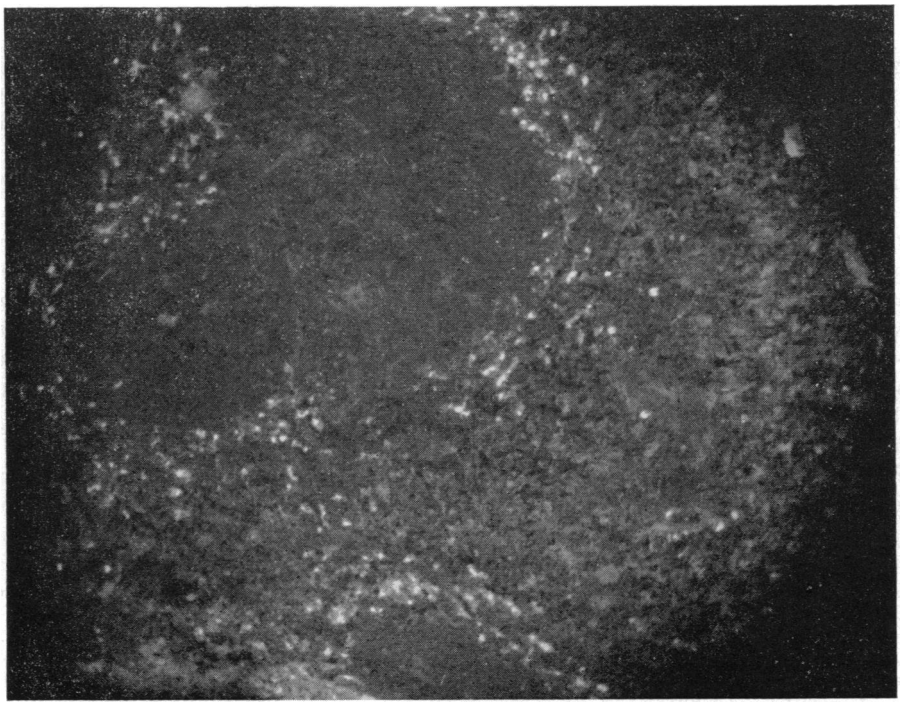

FIG. 4. *Mouse spleen 7 hr after large intravenous injection of the Moscow strain of ectromelia virus; fluorescent-antibody stained section. Most infected cells in a perifollicular position.* ×*100.*

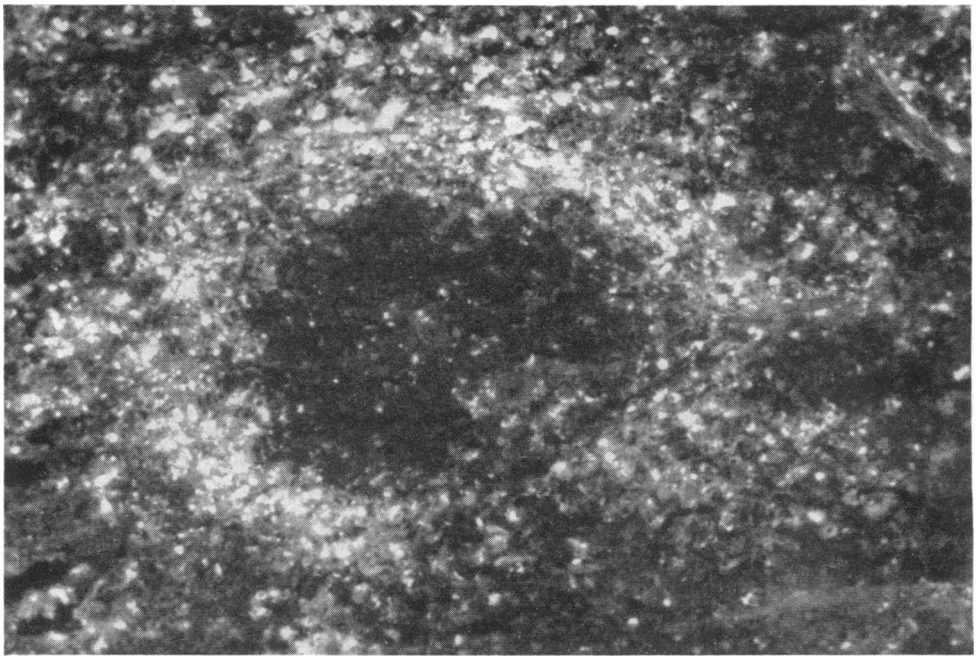

FIG. 5. *Mouse spleen 23 hr after large intravenous injection of the Moscow strain of ectromelia virus; fluorescent-antibody stained section. Outer zone of lymphoid follicle infected, and scattered infected cells in red pulp.* ×250.

be described. The results were broadly the same whether virus was injected intravenously or subcutaneously, but the significant differences make a separate description of each route of infection necessary. After the intravenous injection of large doses of the virulent Moscow strain, the injected virus was phagocytosed by cells, presumably macrophages, lining the sinusoids of the red pulp. The first cycle of growth (7 hr) was principally in these cells, many of which were in a perifollicular position (Fig. 4). At 23 hr, the infection had spread inwards towards the center of follicles. Some follicle profiles were composed entirely of infected cells, and others had an outer ring of infected cells with a central zone containing no more than a very occasional infected cell (Fig. 5). There had also been an increase in infected cells in the red pulp, but the picture was more striking in the follicles. By 31 hr, the infectious process had spread throughout most follicles (Fig. 6), and was accompanied by an extensive destruction of cells with the production of karyorrhetic nuclear fragments. Many of the cells at the edge of follicles which had been infected first had now produced characteristic globular inclusion bodies, (Fig. 7), and this was so for nearly all follicular cells by 48 hr. Mice always died when large doses of virulent strains of virus were injected, but when avirulent strains of virus, or smaller doses of the virulent strains, were given, many mice recovered. In those that recovered, a number of virus-free follicles emerged and grew in size, while antigen disappeared from the infected follicles. It was noted that, although with a given intravenous dose of virus much the same number of foci per unit area of section were initiated in spleen and liver, the splenic foci enlarged much more rapidly. After the subcutaneous injections of virus described below, there was also a more rapid enlargement of splenic foci. No further studies have been made of this difference, which perhaps reflects a basic difference in the cytoarchitecture or in the behavior of cells in the spleen.

The findings after the subcutaneous injections of virus differed from intravenous injections in some respects. Virus now reached the spleen from the blood during the primary viremia, and was likely to be in the form of infected leu-

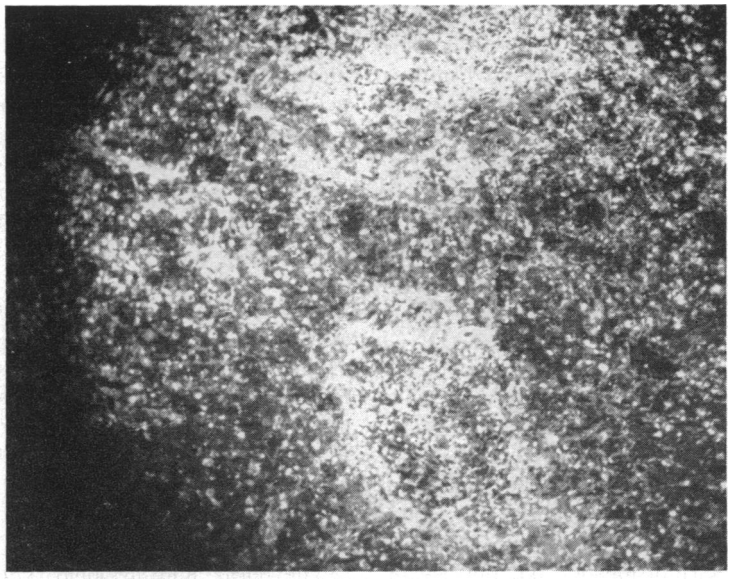

FIG. 6. *Mouse spleen 31 hr after large intravenous injection of the Moscow strain of ectromelia virus; fluorescent-antibody stained section. Confluent infection of two lymphoid follicles and scattered infected cells in red pulp.* ×*100.*

kocytes which were not yet grossly damaged or necrotic. When large doses had been injected, the first infected cells in the spleen were seen after 2 days, and these were either macrophages or lymphoid cells, and were present either in the red pulp or in follicles. The isolated infected cells seen in follicles at this time had clearly migrated there. By the third day, many more foci of infection were present, either as single cells or small groups of cells. At this stage, it should be noted, there were still few, if any, infected cells in the liver. After 4 days, the splenic follicles were severely involved, often by the spread of infection from isolated infected follicular cells, but also by the spread of infection into follicles from the periphery, as was seen after intravenous injections. Infected foci were by now common in the liver, but the infectious process was not as advanced as in the spleen. In these experiments, further observations were made on the emergence of virus-free splenic follicles. At 6 days after large subcutaneous injections of the virulent Moscow strain of ectromelia, all splenic follicles are usually infected, and most of them are destroyed. Some mice are already sick or dead, but in the spleen of those that are still well a few distinct antigen-free follicles are present. These follicles also stand out clearly when such sections are stained with acridine orange,

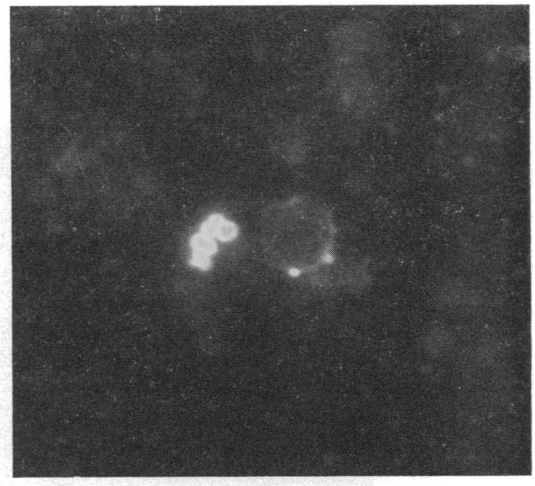

FIG. 7. *Mouse spleen smear 5 days after subcutaneous injection of the Moscow strain of ectromelia virus; fluorescent-antibody stained preparation. Infected small lymphocyte with thin rim of fluorescent cytoplasm and two small fluorescent inclusions. Neighboring large, extracellular inclusion body mass.* ×*1,000.*

because the cells in these follicles have red fluorescent cytoplasm. This indicates that the cells are rich in ribonucleic acid (RNA), and are producing antibody, probably antibody to

ectromelia virus. These new lymphoid follicles arise as a result of mitotic activity, at a time when the growth of virus has depressed mitotic activity in most follicles. This general depression of mitotic activity was shown in an experiment in which mice were injected with tritiated thymidine 5 days after a large subcutaneous injection of the Moscow strain of ectromelia virus. Mice were killed a few hours later, and the spleen was examined autoradiographically. There was a general depression of premitotic incorporation of thymidine into deoxyribonucleic acid (DNA) in follicles, as compared with normal animals.

Thus, after subcutaneous injection, virus tends to reach the spleen in infected cells, and the fact that the spleen is infected earlier than the liver perhaps merely reflects the fact that leukocytes in the blood tend to settle down there rather than in the liver. Infected leukocytes may initiate foci in the substance of follicles, but follicles are also infected from perifollicular regions of the red pulp. The basic features of the process, the early involvement of macrophages, the infection and destruction of lymphoid follicles, and the later emergence of uninfected antibody-producing follicles, occur in the same way as after intravenous injections.

These two sets of experiments show that in mice infected with ectromelia virus there is a race in the spleen between virus growth in follicles and antibody production in follicles. Follicles are being destroyed in the act of responding immunologically, and survival is associated with the development of antibody-producing follicles containing little or no virus. Humoral antibodies are perhaps not of paramount importance in poxvirus infections (67), but, if cellular or other types of immunity are also generated in lymphoid follicles, the results of this race are of great importance. Schell (137), for instance, has shown that the resistance of C57 B1 mice to ectromelia is attributable simply to their more rapid and more efficient immune response after infection.

Lymph Nodes

The local and regional lymph nodes are strategically placed to deal with the flow of lymph from all major regions of the body before returning it to the blood. Especially in tissues like the skin, foreign particles tend to enter afferent lymphatics and reach local lymph nodes. Here, the lymph is monitored by the macrophages lining the peripheral sinuses, and

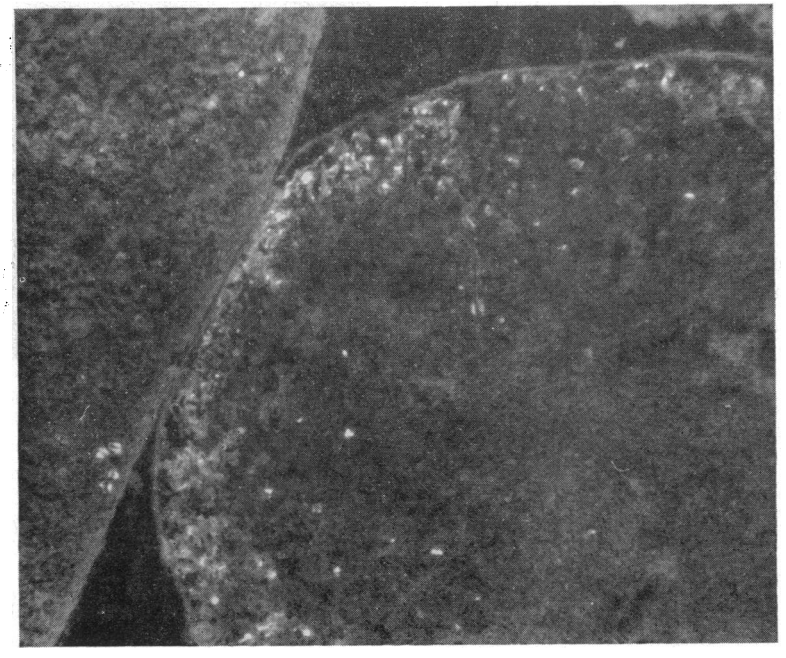

FIG. 8. *Popliteal lymph node of mouse 18 hr after subcutaneous injection of ectromelia virus into the foot; fluorescent-antibody stained section. Infected cells in and adjacent to peripheral sinus.* ×150.

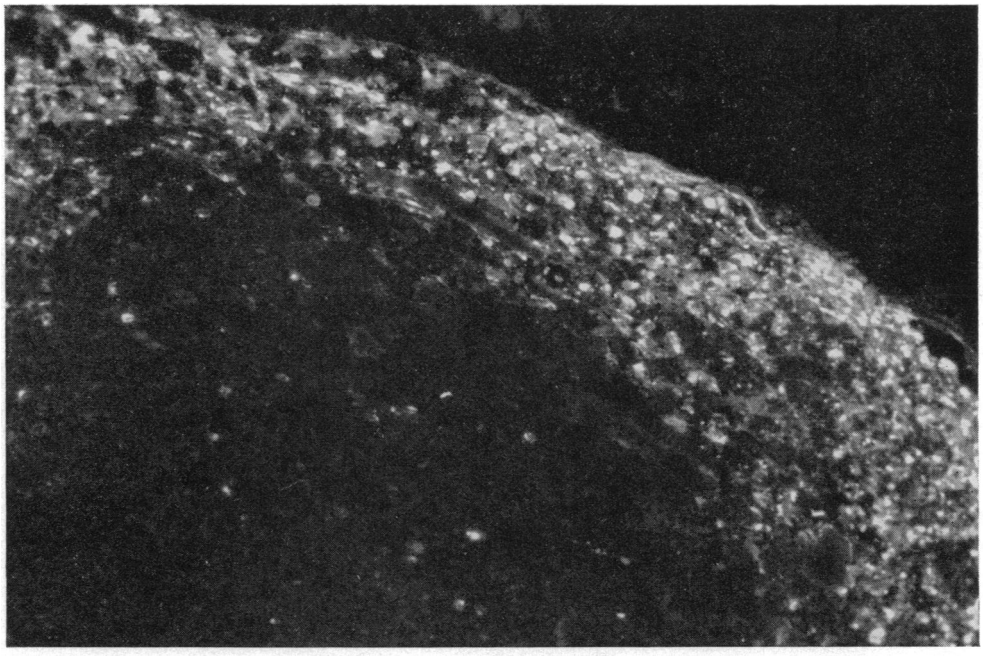

FIG. 9. *Cortical region of popliteal lymph node of mouse 36 hr after subcutaneous injection of ectromelia virus into the foot; fluorescent-antibody stained section. Infected cell area spreading inwards from cortex* ×250.

these cells take up particles present in lymph and thus filter it. Much has been written about the filtering capacity of lymph nodes (169), and the efficiency of filtration depends on the concentration of particles reaching the node, the nature of the particles, the lymph pressure, and other factors. It is probably true that some of the particles entering afferent lymphatics bypass the macrophages and encounter lymphoid cells (48). Most particles, however, are taken up by macrophages, and the results described below suggest that poxvirus particles initially are dealt with in the same way as India ink particles. Liu and Coffin (90) recorded some fluorescent-antibody observations on the early involvement of the cervical lymph node of the ferret after the intranasal instillation of canine distemper virus. Roberts (126) used the fluorescent-antibody method to follow the growth of ectromelia virus in pulmonary lymph nodes of mice after aerosol infection. Apart from this, there is no published work on the histopathogenesis of virus infections of lymph nodes. Further studies of the growth of ectromelia virus in local lymph glands of mice have therefore been made, by use of the fluorescent-antibody technique. It will be seen that the main features of the infection are the same as those described above for the spleen. Large doses of virus were injected subcutaneously, and the first infected cells, seen at 12 hr, were macrophages lining the peripheral sinus of the local lymph node (Fig. 8). The injected virus must have entered lymphatics and thus reached the local lymph node, there to be phagocytosed by macrophages. Roberts (126) showed that lymphatic endothelium itself was infected by ectromelia virus. At later times, the infected cell area had spread towards the hilum of the lymph node (Fig. 9), involving lymphoid follicles just as in the spleen. Occasional isolated infected cells were seen in advance of the main lesion, and it is thought that these may have migrated there after becoming infected. It could be by the migration of such infected cells that infection spreads to the regional lymph nodes and spleen. Still later, by about 2.5 days, nearly all cells in the node were infected, with the development of inclusion bodies and the destruction of cells as described in the spleen. Finally, the node was reduced to an edematous reticular framework containing inclusion bodies and an occasional intact cell (Fig. 10). Peripheral sinuses were

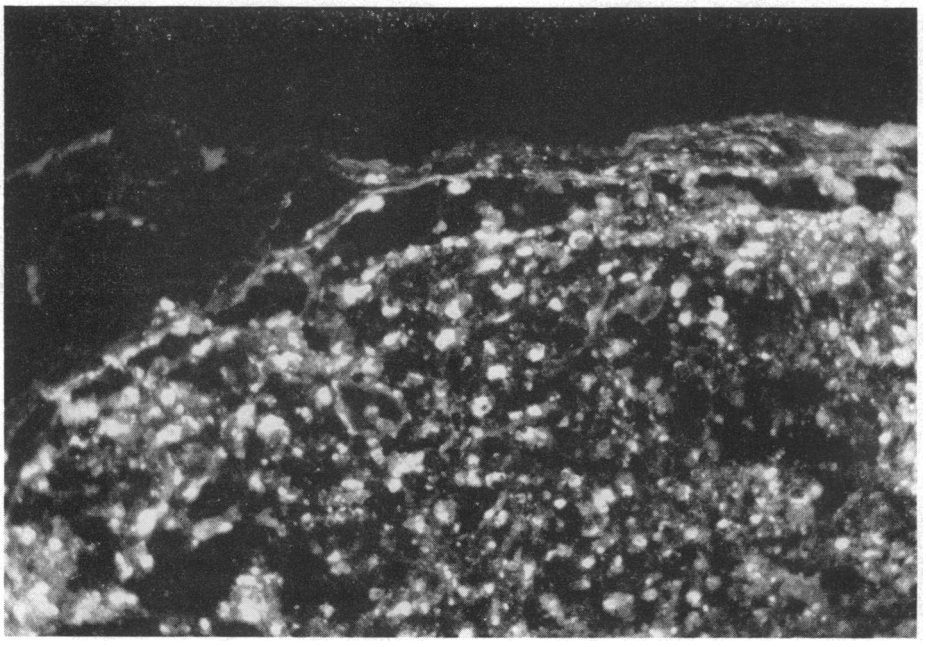

FIG. 10. *Cortical region of popliteal lymph node of mouse 3 days after subcutaneous injection of ectromelia virus into the foot; fluorescent-antibody stained section. Edema of peripheral sinus, inclusion bodies, infected cell debris.* ×*250.*

dilated, and it is unlikely that there was at this stage a free circulation of lymph through the node. This is the stage of infection, with dilation of the peripheral sinuses and afferent lymphatics ("lymphatic blockade"), which was also described by Roberts (126).

Thus, in the local lymph node, as in the spleen, lymphoid cells are destroyed while the immune response is in progress. In the local lymph node, however, this race between the immune response and the destruction of immunologically active cells is less critical, because there are further lines of host defense in the form of the regional lymph nodes and the spleen.

In the experiments described above, where large amounts of virus were injected subcutaneously, the injected virus entered afferent lymphatics within a very short time and was phagocytosed by the macrophages in the peripheral sinuses. Ectromelia virus has been demonstrated in local lymph nodes within a few minutes of such injections. When a small dose (100 pfu) was given subcutaneously, the local lymph node was not infected until 21 hr (Mims, *unpublished data*). After such doses, and probably in natural infections, local lymph nodes are likely to be infected by the movement of infected cells. Roberts (126) showed that, in mice infected with ectromelia virus by aerosol, the pulmonary lymph nodes were not involved until the third day, and this was by the movement of infected macrophages into the nodes.

It is not only local lymph glands draining the site of infection that are involved in mice infected with ectromelia virus. After intravenous injection, for instance, foci appear in lymph nodes throughout the body, but these foci are probably initiated by infected cells which enter nodes from lymphatics or from the blood, because they arise anywhere in the node (Fig. 11). Much has been written about the possible spread of virus infection through the body by way of the lymphatic system (169), and the infection of tissues by the movement of infected lymphoid cells was clearly envisaged by Yoffey and Sullivan (168). Are lymph nodes important strongholds of host resistance, or are they merely centers for the dissemination of the infection? A short discussion of this topic will follow, together with a survey of the very limited experimental evidence available.

It is generally thought that subcutaneously injected ectromelia virus enters the blood via the

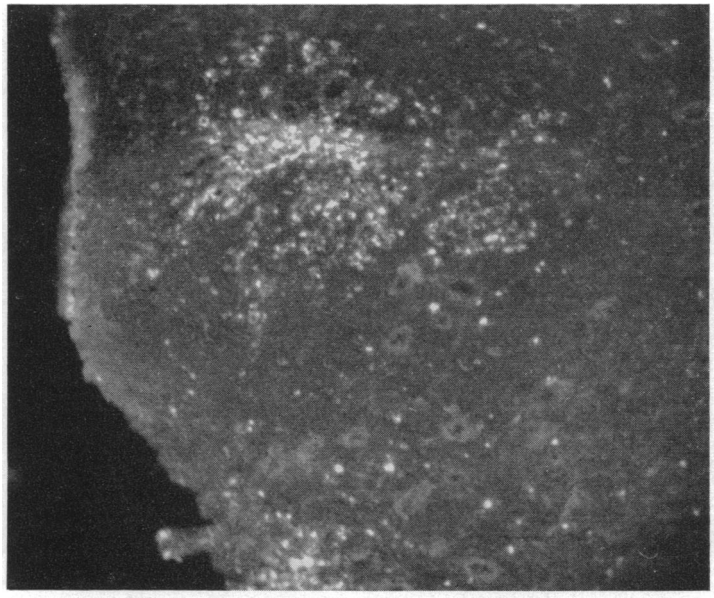

FIG. 11. *Local lymph node of mouse 2 days after intravenous injection of the Moscow strain of ectromelia virus; fluorescent-antibody stained section. Infected cell area near center of node.* ×*150.*

lymphatic system, and then infects viscera (55). This is probably true for vaccinia virus, because Yoffey and Sullivan (168), who infected rabbits intranasally, found virus from the twelfth hour onwards in cervical lymph in association with lymphoid cells, virus having traversed at least one lymph node. The fluorescent-antibody observations of Liu and Coffin (90) on ferrets infected intranasally with canine distemper virus also suggest that virus reaches the blood after infecting the cervical lymph node. There is good evidence that tick-borne encephalitis virus enters the blood of mice via the lymphatic system. Malkova and Frankova (95) injected this virus subcutaneously into the forelimb of mice after the removal of the regional lymph nodes. There was a significant delay in the time at which viremia was detectable, and death occurred on the average 2 days later than in control animals. In further experiments with sheep, Malkova (96) obtained evidence that virus entered the blood only via the thoracic lymph duct. In the same way, ectromelia virus could enter the blood of mice via the regional lymph nodes and the thoracic duct. If, as in vaccinia in rabbits, virus was in mobile infected cells, these need not necessarily infect the nodes through which they passed. This is consistent with the observation that, after the subcutaneous injection of ectromelia virus, the primary viremia, as judged by infection of the spleen, occurs before the infection of all lymph nodes leading from the injection site to the thoracic duct (Mims, *unpublished data*). Alternatively, ectromelia-infected cells could enter the blood directly from lymph nodes without passing through the thoracic duct. Indeed, if a macrophage or lymphoid cell infected in the dermis migrated to the local lymph node and there entered the blood before it became damaged or yielded virus, a viremia could be initiated without actual infection of lymph nodes. In any case, ectromelia probably reaches the blood to some extent via the lymphatic system. Thus, when large doses of ectromelia were injected in small volumes (0.005 ml) into the toe of mice and the limb was amputated 1 hr later, mice were nevertheless infected and died. The same result was obtained when virus was scarified into the foot. The amputation left the popliteal node intact, and virus was in effect introduced into this node, which is known to contain virus within a few minutes of such infections. Virus spread through the mouse from this initially infected lymph node. In control mice whose uninfected foot was amputated, the average survival time was shorter. This is doubtless because virus spread through the body not only from the initially infected lymph node but also, at a later

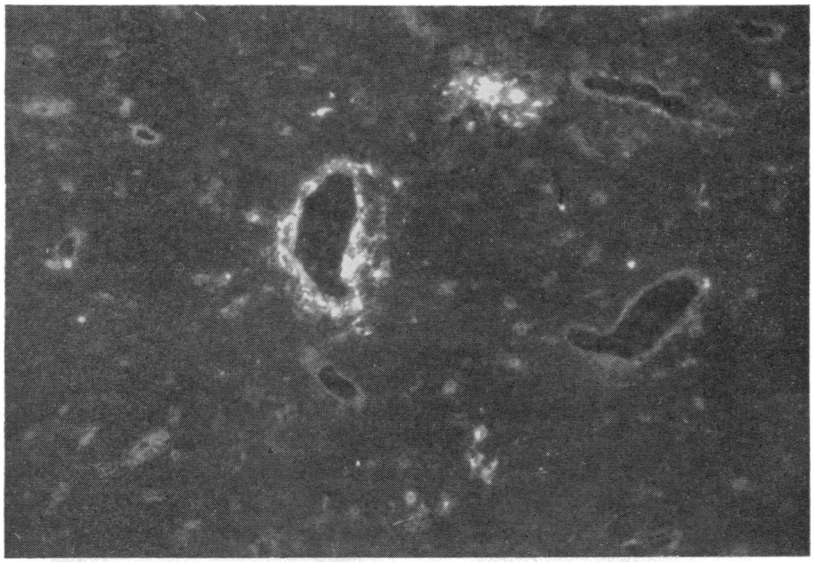

FIG. 12. *Mouse thymus 2 days after intravenous injection of the Moscow strain of ectromelia virus; fluorescent-antibody stained section. Foci originating from blood vessels.* ×200.

stage, from the sites of virus growth in the skin and subcutaneous tissues at the injection site. A similar experiment with vaccinia virus in rabbits was performed by McMaster and Kidd (94).

The following experiments make it possible to suggest that ectromelia virus enters the blood directly from tissues as well as via the lymphatic system. Parasacral, inguinal, iliac, and pararectal lymph nodes were removed from mice 3 days after the injection of India ink into the tail. The following day, a moderately large dose of ectromelia virus was either scarified or injected subcutaneously in a small volume (0.01 ml) into the end of the tail. In two experiments, 20 mice were used, and these mice died with an average survival time not detectably different from control sham-operated mice. It seems likely that virus entered blood vessels directly from dermal tissues by the growth of virus or the passage of infected cells through vessel walls.

The poxviruses are only one group of a large number of viruses infecting lymphoid tissue. In poliovirus (24, 147) and arthropod-borne (95, 96, 97, 148, 159) and other virus infections (90), for instance, the infection of lymphoid tissue may play an important part in the disease process. With the evidence now available, however, it is difficult to say how often lymph nodes are centers for the dissemination of virus rather than important host strongholds where infection is limited and localized while the immune response proceeds. Probably lymph nodes can behave in both ways, for there are two important things to remember. First, the movement of lymphoid cells from the infected area through the body is probably an inevitable or even an important part of the generalized immune response. Second, if a virus grows in and destroys lymphoid cells, as does ectromelia, a response which under other circumstances is protective may then become less of an advantage. All viruses, therefore, might not behave in the same way.

Other Lymphoid Tissues

It is known that poliovirus, for instance, infects the subepithelial lymphatic tissues comprising the tonsils and Peyer's patches (24), and that there are viruses which destroy the thymus gland of newborn mice (133), but, in these cases, one does not know which cells are infected and the infection cannot be described histologically. Such information again comes from unpublished work on ectromelia virus infections of mice where the fluorescent-antibody technique can be used.

Lymphoid cells throughout the body are readily infected by ectromelia virus, and following most infection procedures it is those in the lymph nodes, spleen, and Peyer's patches (Mims, *unpublished data*) that are most commonly involved, either by free virus particles or by the

movement of infected cells. Lymphoid cells in the peritoneal cavity or the thymus gland, on the contrary, are infected less often. Peritoneal lymphocytes, however, are infected on a large scale when virus is introduced directly into the peritoneal cavity. Late in the disease, too, occasional foci of infection in the thymus develop from small blood vessels (Fig. 12), showing that thymocytes, as well, are infectable if virus has access to them.

Immune Response

In lymphoid tissue throughout the body, macrophages occur in intimate relation with lymphoid cells, and virus particles reaching this tissue tend to encounter macrophages before lymphoid cells (see above). Even if the encounter with macrophages takes place elsewhere in the body, the macrophages can then migrate to lymphoid tissue as was described above for the spleen, and by Roberts (126) for pulmonary lymph nodes. Often, therefore, macrophages which have phagocytosed virus particles find themselves next to lymphoid cells, and the immune response will be discussed with particular reference to these macrophage-lymphoid cell associations.

Infecting viruses could induce antibody formation in two ways: first, by entering antibody-producing cells without causing a damaging infection; and, second, by entering macrophages which then handed on antigens or messages about antigens to the antibody-producing cells. It is difficult to avoid the conclusions that macrophages are involved in the initiation of the immune response to viruses because of their primary importance in the phagocytosis of foreign materials. It has been suggested that macrophages turn into antibody-producing cells (54), and that macrophages themselves produce antibody (92). It has also been suggested that macrophages prepare antigen for antibody-producing cells (58, 129, 134), and the evidence for this has sometimes been good. In lymphoid tissue, where macrophages which have taken up virus particles are often next to the lymphoid cells which are involved in the immune response, there would be numerous opportunities for a handing over of antigen or messages about antigen. The occurrence of macrophage-lymphocyte associations during the response to nonviral antigens was described by Sharp and Burwell (141). The observations which follow describe more fully the opportunities for this handover which occur in ectromelia virus infections, although there is, at the moment, no evidence that it ever in fact occurs.

Many cells, late in infection with ectromelia

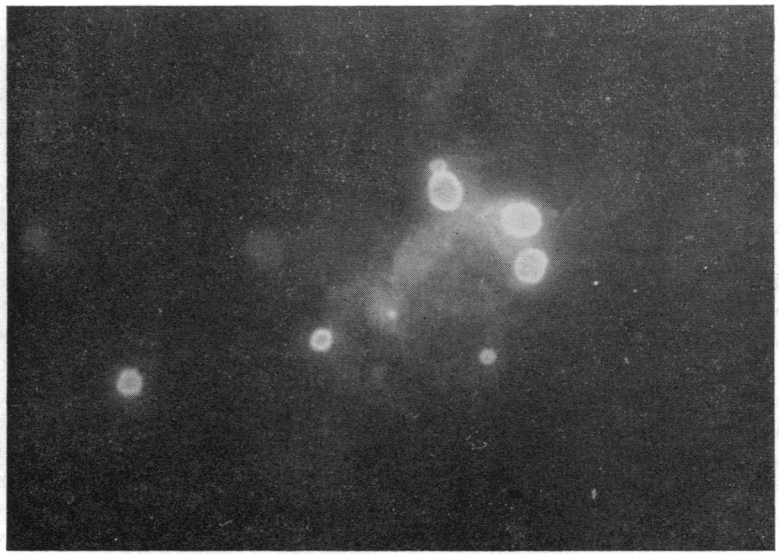

FIG. 13. *Spleen smear from mouse 5 days after subcutaneous injection of Moscow strain of ectromelia virus; fluorescent-antibody stained preparation. Infected macrophage with fluorescent cytoplasm, containing fluorescent globular inclusions. Neighboring globular inclusions perhaps released from the macrophage.* ×*1,000.*

virus, contain antigen in the form of spheres which are clearly visible as rings by fluorescent-antibody staining (Mims, *unpublished data*). In infected mice, these rings are particularly prominent in the lymphoid tissues (Fig. 13). They are often liberated from cells and are seen free in the blood late in infection. When examined by electron microscopy, some, at least, of the rings do not contain virus particles. Macrophages produce rings in vitro, and it has been shown by combined fluorescence microscopy and autoradiography that rings probably do not contain viral DNA. Macrophages, then, segregate non-infectious antigen into spherical inclusions. These, if they were taken up by lymphoid cells, would seem to be the ideal postulated antigen prepared in macrophages for antibody-producing cells. Unfortunately, at the moment this is no more than an intriguing possibility, and the biological significance of rings, if any, is unknown.

As was pointed out above, macrophages, since they monitor the main body compartments, encounter virus particles at an early stage after infection. Whether or not they are in close association with lymphoid cells, they have an admirable opportunity to express the immune response; a discussion of this topic will follow.

The immune response would be expressed by macrophages in a different behavior to virus particles, and this might be because the macrophages themselves were different or merely because virus particles were coated with specific antibody. Acquired resistance to many virus infections occurs in the absence of humoral immunity (67), and the delayed-type hypersensitivity reaction, as an index of cellular immunity, has been found in almost every virus disease where it has been carefully looked for. In immune animals, therefore, macrophages could themselves be immune. They could also respond in an immune fashion to antibody-coated virus particles which they might inactivate more readily than do normal macrophages. Ginder (64) showed that immune cells from peritoneal exudates, lymph nodes, liver, spleen, and buffy coat of fibroma-recovered rabbits increased the neutralizing power of immune serum, whereas normal cells had a variable effect. This could have been because of a difference in the behavior of immune macrophages, but it was thought that it might have been due to a cellular transfer by lymphoid cells of the ability to produce antibodies.

Until recently, there had been no very convincing evidence as to the behavior of macrophages in animals immune to virus infections (59, 146). The findings with ectromelia virus now to be described illustrate the importance of antibody and indicate that in this instance macrophages themselves are not specifically immune. When partially purified ectromelia virus was injected intravenously to hyperimmune mice, the inoculum was taken up by liver and spleen macrophages as in normal mice, and could be located by the fluorescent-antibody technique. Antigen faded and, when moderate doses were injected, failed to reappear. Virus premixed with immune rabbit serum was taken up in the same way in normal mice and could be demonstrated in liver macrophages by fluorescein-labeled antiserum to rabbit gamma-globulin. The rabbit gamma-globulin antigen slowly faded, and ectromelia failed to appear (grow) in the cells.

It is concluded, therefore, that virus coated with specific antibody is phagocytosed by macrophages which are then able, perhaps because of this antibody, to digest the virus particles which would normally have infected them. When, however, peritoneal macrophages from ectromelia hyperimmune mice were very thoroughly washed and maintained in vitro (Roberts, *in preparation*), they were infected no less readily than normal macrophages. The result was the same when immune serum was present. Macrophages from recently hyperimmunized mice were, if anything, more readily infected than were normal macrophages, and this was attributed to differences in the morphology and phagocytic activity of immune macrophages. These experiments argue strongly against any specific differences in the reaction of immune macrophages to virus. Recently, Cohn (40) showed that the degradation of P^{32}- and C^{14}-labeled bacteria proceeded in the same way in macrophages from normal and immune rabbits. On the other hand, Maral (98) found that macrophages from myxoma-immune rabbits produced very little infectious virus compared with normal macrophages, when infected in vitro. Such a result would only be acceptable if there was no doubt that all traces of antibody had been removed from the cells before infection, because Roberts

(*in preparation*) showed that antibody still reduced the infection rate in macrophages when diluted 1:1,000.

VIRUS INFECTIONS OF OTHER ORGANS AND TISSUES

The organs and tissues discussed in this section were selected either because of their intrinsic interest or because of the information about them obtained from recent experiments, published and unpublished. In no case has a comprehensive account been attempted; rather, the opportunity has been taken to illustrate the use of the fluorescent-antibody method, draw attention to the part played by macrophages, and discuss some neglected topics.

Peritoneal Cavity

The peritoneal cavity of mice contains a thin film of liquid between serosal surfaces, and more than 10^6 cells can be washed out from this cavity with physiological fluids. This is a most convenient and satisfactory source of macrophages for in vitro studies. While up to 25% of these cells are certainly macrophages and up to 65% are lymphocytes, there are usually a number of cells of intermediate type, many of which, like the macrophages, are capable of taking up intraperitoneally injected particles. The presence of this lymphocyte-macrophage spectrum, in conjunction with experimental findings, has been taken as evidence that peritoneal lymphocytes turn into macrophages (46, 142). Macrophages monitor peritoneal fluid, and intraperitoneally injected particles are phagocytosed by macrophages, both free in the peritoneal fluid and attached to the serosal peritoneal surfaces. Serosal cells are only weakly phagocytic and take up small amounts of carbon or thorotrast. Particles also leave the peritoneal cavity, enter the subdiaphragmatic lymphatics (61), and reach the retrosternal lymph nodes, but they have no direct route of entry into the blood except via lymphatics.

Not much is known at a cellular level of the pathogenesis of virus infections of the peritoneal cavity, and the course of events after the injection of virulent strains of ectromelia virus intraperitoneally into mice will be described, as studied by the fluorescent-antibody technique (Mims, *unpublished data*). The injected particles which are taken up by macrophages grow in them, and thence infect other macrophages. The retrosternal lymph nodes are infected by 8 hr when large doses of virus are injected, perhaps by the inoculated particles or perhaps by the movement of infected cells, and a small amount of virus, probably cell-associated, is present in the blood within a few minutes (138). As the infection spreads in the peritoneal cavity, more and more macrophages, both free and on the mesentery, become infected, together with lymphocytes and occasional serosal cells. By the time mice are sick, 2 days after a large virus injection, there has been a large-scale destruction of peritoneal macrophages. Normally about 10^6 macrophages can be readily obtained in peritoneal washings, and in vitro these cells become firmly attached to the glass within an hour. In mice sick after the intraperitoneal injection of ectromelia virus, however, few if any intact peritoneal macrophages can be recovered. Fluid is often present in the peritoneal cavity, and it contains cell debris, virus particles, inclusion bodies, and occasional cells of the lymphocyte-macrophage transitional class. Carbon injected intraperitoneally to these mice remains largely extracellular.

It was shown for ectromelia virus (138) that the intraperitoneal route of infection is the most lethal of all. A dose of a virulent strain of virus which just infects by the subcutaneous route kills when injected intraperitoneally. In attempting to account for this intraperitoneal lethality, a number of experimental observations were made. First, the possibility that there was a direct invasion of abdominal organs from the peritoneal cavity, as indicated for herpes simplex in suckling mice by Beswick (19*a*), was investigated. Fluorescent-antibody studies, however, showed that virus did not spread into the liver, spleen, adrenals, kidney, or Peyer's patches from the serosal surface of the peritoneum. The liver and spleen were, in fact, infected later than after the less lethal intravenous route of injection. Furthermore, there was no more involvement of heart, pleura, or pericardium than after other routes of infection. These observations alone would suggest that the balance between growth in target organs and the immune response should be weighted in favor of host survival, so other more important factors must be operating in the

other direction. It was thought that the antimicrobial defenses of the peritoneal cavity of mice might be weakened as a result of ectromelia infection, and bacterial cultures were made of peritoneal fluid and blood. It was found that the peritoneal cavity was often invaded by bacteria, particularly *B. proteus*, at a late stage of the disease after intraperitoneal infection. Bacteremia was uncommon.

In spite of these observations, antibiotic treatment was found to have no effect on intraperitoneal lethality as determined by intraperitoneal titrations. Mice fed large amounts of tetracyclines, which develop the enlarged caecum seen in germ-free rodents (68), gave the same intraperitoneal end points as did control mice. These observations do not exclude secondary invasion by microorganisms as a factor accounting for the lethality of the intraperitoneal route of infection, because pleuropneumonia-like organisms, for instance, were not investigated, nor was the possibility that the invading bacteria were resistant to tetracyclines. Nevertheless, other causes for the lethality of the intraperitoneal route were sought, and the interesting possibility that there is an alteration in the host immune response after intraperitoneal infection was considered.

Schell (138) showed that C57 B1 mice, whose resistance to ectromelia is attributable to a superior immune response, are as susceptible as other strains of mice to intraperitoneal infection. Schell's experiments suggest, too, that intraperitoneally infected mice produce antibodies to ectromelia as effectively as do mice infected by the less lethal subcutaneous route. An explanation of intraperitoneal lethality was therefore sought in terms of a change in the ability to develop cellular immunity. Destruction of macrophages and lymphocytes is a distinctive feature in infection with virulent strains of ectromelia virus, and, although this involves cells lining blood sinusoids and those in lymphatic tissue whatever the route of inoculation, the macrophages and lymphocytes in the peritoneal cavity are usually spared, except after intraperitoneal inoculation. The milk spots lining the peritoneal cavity of mice are a source of macrophages (Mims, *unpublished data*), and it is possible that the peritoneal macrophages or lymphocytes contribute in some way to the host defense mechanisms, conceivably by migrating to the sites of visceral growth of virus and replacing cells which have been destroyed. This would not occur after intraperitoneal injections, because there is a primary destruction of peritoneal cells. Mice might then die in spite of the delay in the infection of target organs.

Thorotrast was used in an attempt to remove peritoneal macrophages in mice infected subcutaneously with ectromelia virus. An intraperitoneal injection of 0.4 ml of thorotrast is not lethal and results in a 99% destruction of peritoneal macrophages by 18 hr. It was found that an otherwise nonlethal subcutaneous dose of ectromelia became lethal when mice were given such an intraperitoneal dose of thorotrast on the sixth day after infection. Small doses of thorotrast were ineffective, and it was found that with such doses there was a rapid cellular recovery in the peritoneal cavity so that large numbers of young macrophages were present by 24 hr. The result of the experiment, however, is not easy to interpret. Does ectromelia make mice susceptible to a sublethal dose of thorotrast, or does thorotrast make them susceptible to a sublethal virus infection? It is not possible to decide about this, and it is still no more than a possibility that intraperitoneal infections with ectromelia virus are particularly fatal because of the destruction of peritoneal cells.

Connective Tissues

Very little is known about the growth of viruses in connective tissues, and in this section some of the recent fluorescent-antibody studies on the growth of poxviruses in subcutaneous tissues will be discussed. The experimental findings are about the growth of viruses at the site of introduction into the skin, and it will be seen how macrophages play an important part in the infectious process.

Roberts (127) made a careful study of the growth of ectromelia virus after scarification into the skin of mice. Infection was not initiated very readily unless scarification penetrated below the epidermis, and the first cells infected, as determined by fluorescent-antibody examination of serial sections at 8 hr, were more likely to be in the dermis than in the epidermis. The primarily infected dermal cells were believed to be macrophages, and it was from these cells that the infectious process appeared to spread. Spread in the dermis initiated "island foci" of epidermal

infection in advance of the main, more slowly expanding epidermal focus, and spread into lymphatics initiated infection in local lymph nodes. Such spread could involve the movement of free virus, the stepwise invasion of stationary cells, or the movement of mobile infected cells. Free virus almost certainly enters afferent lymphatics, and evidence for the movement of infected macrophages into local lymph nodes was obtained by Roberts (126) for the spread of ectromelia virus from lungs to pulmonary lymph nodes of mice. Thus, in these experiments epidermal cells could be infected *ab initio*, but the first infected cells in the underlying skin were probably macrophages. Macrophages were probably important in the spread of infection in the dermis and perhaps played a part in the infection of local lymph nodes.

Macrophages in connective tissues (histiocytes) monitor these tissues, taking up extravasated red blood cells or subcutaneously injected India ink, and it is thought that infecting viruses are dealt with in the same way as other foreign particles. Tompkins and Grillo (156) pointed out that the resting reticuloendothelial cells of the subcutaneous tissues are for many hours the only mononuclear cells available for the phagocytosis of invading organisms, and Ledingham (87, 88) drew attention to the importance of dermal reticuloendothelial cells in the response of rabbits to intradermally injected vaccinia virus. Macrophages are infected (Roberts, *unpublished data*), and are certainly important in the growth of myxoma virus in the skin of rabbits; indeed, myxoma cells themselves are perhaps derived from connective tissue macrophages (1).

One consequence of the very early virus-macrophage encounter in connective tissues is that host susceptibility or insusceptibility might well be established at this stage of infection. If virus is phagocytosed and destroyed in macrophages, there is little opportunity for the spread of infection in subcutaneous tissues. Should a connective tissue cell other than a macrophage be infected, then as soon as this cell is damaged or releases virus, local macrophages, in exercising their phagocytic function, would take up and destroy virus particles. Even if free virus reached local lymph nodes, it would be likely to be phagocytosed by the macrophages of the peripheral sinuses (see above). On the other hand, macrophages would be less important if lymphoid cells were infected in subcutaneous tissues and then migrated into lymph nodes. Also, they would not have a determining influence in the case of viruses growing exclusively in epidermal cells, although they might ensure that such infections were restricted to epidermal cells.

Bone Marrow

Almost nothing is known of virus infections of the bone marrow, and in the following short discussion of the subject a few fluorescent-antibody observations are reported, and the possible importance of bone marrow macrophages pointed out.

In fluorescent-antibody studies of mice infected intravenously with ectromelia virus, only occasional small foci of infection were seen in the bone marrow. Marrow foci were not substantially different in the case of rabbits infected intravenously with large doses of rabbitpox or myxoma viruses (Mims, *unpublished data*). Although the macrophages lining the sinusoids in the bone marrow monitor the blood, they are quantitatively far less important than those in the liver and spleen. Only a small proportion of circulating virus particles, therefore, would localize in the bone marrow, and as a result bone marrow foci are infrequent.

Localization in the bone marrow might be a more important feature in infection with leukemia viruses. In malignant panleucopenia of cats (70), the virus probably grows in the bone marrow to cause aplasia of nearly all blood-forming cells. Macrophages might play an important part in such diseases, particularly if marrow lesions were initiated by free rather than by cell associated virus, and, in electron microscope studies of the bone marrow of chickens infected with erythroblastosis virus, Iwakata (81) recorded the presence of presumed virus particles in microphages.

Cerebrospinal Fluid (CSF)

The CSF is thought to be important in virus diseases for three reasons: first, because viruses crossing the blood-CSF barrier may cause meningitis; second, because viruses in the CSF could then enter and grow in central nervous tissue; and, third, because of its role in experimental intracerebral injections.

When India ink is injected intracerebrally into

mice, it is distributed in the CSF spaces comprising the ventricles, subarachnoid, and Virchow-Robin spaces (108), and moderate numbers of carbon-containing macrophages are seen, mainly in the subarachnoid spaces, a few hours after the injection. It is concluded that macrophages normally monitor the CSF and that virus particles would tend to be phagocytosed like the carbon particles in India ink. Although the ependymal cells lining the ventricles are not demonstrably phagocytic, virus particles in the CSF would also have an opportunity to infect these cells.

Some viruses grow in the cells lining the CSF spaces but are unable to infect parenchymal cells. Ectromelia virus, several strains of vaccinia virus (108), the MEL strain of influenza virus (109), and probably LCM virus are in this category. Ependymal cells and cells lining the subarachnoid and Virchow-Robin spaces are infected after intracerebral injection of these viruses, and, although there may be occasional spread to subependymal cells, there is no invasion of the brain parenchyma. In contrast to this, the neuroadapted NWS strain of influenza virus, which undergoes the same preliminary growth in the cells lining the CSF, then invades parenchymal tissue. Arthropod-borne viruses which grow in the brains of mice after intracerebral injection perhaps reach the parenchyma by the same route. The fluorescent-antibody method, however, gave no evidence that intracerebrally injected Murray Valley encephalitis virus (108) or Semliki Forest virus (Mims, *unpublished data*) grew in ependymal cells or cells lining the subarachnoid spaces before infecting parenchymal cells. Possibly virus particles pass through these cell layers without growing in them, in the same way that certain viruses pass through the macrophage lining of liver sinusoids and infect hepatic cells (see above). There was no evidence that these viruses invaded the brain parenchyma by way of the cells lining the needle track.

Meningitis occurs in a number of generalized virus diseases, and the most likely way in which virus particles enter the CSF from the blood is by passing through or growing through the choroid plexuses which constitute the principal blood-CSF barrier. Coxsackie and ECHO viruses, for instance, are commonly isolated from the CSF in patients with aseptic meningitis. In patients with a meningitis due to poliovirus, there is a cellular and humoral effusion into the CSF, but it is uncertain whether this is due to virus infection of the CSF, because isolations from the CSF are rare.

Once in the CSF, viruses capable of crossing the pia have a chance to infect central nervous tissue, and many coxsackie and adenoviruses would have this chance. Poliovirus, if it were present in the CSF could probably cross the CSF-brain barrier because Schaeffer et al. (136) and Hurst (79) showed that virus could invade the brain and produce typical symptoms and disease when introduced very carefully into the CSF of the monkey without damage to the underlying pia.

Lung

In this short discussion of virus infections of the lung, particular attention will be paid to the initiation of infection in relation to the mechanisms available for the removal of foreign particles from the respiratory surfaces.

A mucociliary "blanket" covers much of the surface of the respiratory tract; foreign particles deposited on this surface are entrapped in mucus and borne upwards by ciliary action. Distal to the respiratory bronchioles, the mucociliary blanket is absent, and here macrophages play an important part in the uptake of inhaled particles (14, 130, 167). Electron microscopic studies of the septal macrophages in the alveoli of mice were made by Karrer (85), who also described the uptake by these cells of intranasally administered carbon particles. The function of macrophages in removing inhaled particles of dust and soot in town-dwelling man is well documented, as is the role of macrophages in the uptake of inhaled tubercle bacilli and other microorganisms. Macrophages occur less regularly on mucociliary areas of the respiratory tract, and these have perhaps entered mucociliary regions from below and are being carried passively upwards towards the trachea and pharynx.

Thus, virus particles entering mucociliary regions of the lower respiratory tract are likely to be entrapped in mucus and carried upwards, whereas those deposited distal to these regions are likely to have a primary encounter with macrophages.

The fluorescent-antibody observations of Roberts (126) on mice infected by aerosol with ectromelia virus will now be discussed. Roberts

serially sectioned lung segments to detect the first infected cells, and found that these were as likely to be macrophages as mucosal cells. It was macrophages, moreover, that carried virus to the pulmonary lymph nodes. Clearly, macrophages did not prevent the infection of mucosal cells, but they did play a part in the genesis of infection in the lower respiratory tract, and probably carried virus and antigen to immunologically active sites.

In the case of influenza virus in mice, fluorescent-antibody studies showed that intranasally administered virus infected mucosal cells more readily than did ectromelia (Roberts, *personal communication*). Macrophages were present in infected areas, and some of them were possibly infected. In fluorescent-antibody studies of ferrets infected with influenza virus, Liu (89) saw antigen-containing macrophages in alveoli and also in mediastinal lymph nodes and nasal smears.

Thus, macrophages take up virus particles deposited distal to the mucociliary regions of the lower respiratory tract, carrying antigen to the pulmonary lymph nodes and perhaps the spleen. They also phagocytose infected cell debris and, because of their position in alveoli, control the entry of virus into the blood. Those entering the mucociliary regions are probably borne upwards to the trachea and oropharynx.

Alimentary Canal

Many viruses are infectious by mouth, but almost nothing is known about the way in which infection takes place. Where do virus particles enter the body, and what cells are involved? There must be a primary encounter between virus particles and the cells lining the alimentary tract, either the epithelial cells or the lymphoid cells which are very close to the surface in the oropharynx and Peyer's patches. Although no longer fashionable, a primary infection of nerve endings in the gut has also been suggested (53). The first opportunity for infection occurs in the oropharynx, and evidence points to the early involvement of the lymphatic tissue in the oropharynx after oral infection with poliovirus (24). There is no evidence as to whether other viruses initiate infection in the oropharynx, nor whether primary infection lower down the alimentary canal is ever important. Below the esophagus, viruses must run the gauntlet of digestive enzymes, mucus, acid, and bile, and, although enteroviruses are resistant to these materials, this could be because they have to be excreted into the gut to infect new hosts, not because resistance is necessary for the act of infection. Our almost complete ignorance of the method of infection by enteroviruses may be lightened when technical difficulties with the fluorescent-antibody method in infected animals are overcome.

If infecting virus particles are able to enter host tissues directly by merely passing across or being carried across the layer of cells lining the alimentary tract, there would be no need for preliminary growth in these cells. The primary process of infection would then be a passive one. Indeed, if all small particles were treated in this way, any virus entering the alimentary tract might enter host tissues and have the opportunity to grow in them. There are a few experimental findings which have a bearing on this possibility. Sanders and Ashworth (135) administered polystyrene latex particles to adult rats by stomach tube, and 1 hr later the particles were seen by electron microscopy in vesicles in intestinal epithelial cells, and in intercellular spaces; later, they were present in submucosal lymphatics and in the liver. Clark (34), however, was only able to demonstrate the uptake of proteins and colloidal materials in suckling rats and mice. Mims performed experiments (*unpublished data*) in which adult rats in restraining cages with thoracic duct fistulae drank saline containing large quantities (10^{11} pfu) of T_7 bacteriophage. Infective bacteriophage appeared in thoracic duct lymph within 20 min, and nearly 1,000 pfu were collected during the next 1.5 hr. These experiments offer convincing confirmation of the work of Sanders and Ashworth (135). Bacteriophage particles might have passed immediately into Peyers patches, or, more probably, entered submucosal lymphatics and thus reached thoracic duct lymph. Although only a small fraction of the ingested bacteriophage entered host tissues, the fact that an occasional particle did so is of some significance. The minimal oral infective dose of enteroviruses may not be very large, but the ease with which particles pass across intestinal epithelium might well depend on such things as the type of food eaten, the stage of digestion, and the presence of other microorganisms.

Similar experiments with mice were negative. Normal adult mice were allowed to drink large quantities of type I poliovirus (Mims and Howes, *unpublished data*) or T_7 bacteriophage (Mims, *unpublished data*), but infectious virus could not be detected in mesenteric lymph nodes, blood, or liver up to 2 hr later. Virus did, however, occasionally reach these tissues when introduced by stomach tube, and although natural infection could conceivably involve minute injuries or denudations of intestinal epithelium, it seems that misleading results may be obtained when intestinal epithelium is subjected to the trauma of a stomach tube. The significance of these negative experiments with mice is, however, questionable, because in the rat thoracic lymph duct experiments more than 100 times as many particles were ingested.

All such experiments would be misleading if, as is possible, natural pathogens behave differently from the marker particles. Gerichter (63) found *Salmonella typhi* in the blood of mice 2 to 3 min after the presumed atraumatic oral administration of very large numbers of bacteria.

MICROPHAGES (POLYMORPHS) AND VIRUSES

Polymorphs do not feature very prominently in the pathological lesions produced by viruses, and as a rule the cellular response involves large and small mononuclear cells. Polymorphs leave the blood to enter acutely inflamed tissues, especially in bacterial infections, and it is generally assumed that they play no part in the lesions produced by viruses. This may be true, but almost no studies have been made of the relation between the phagocytic activity of polymorphs and macrophages since those of Lucke et al. (91); a few experiments on virus-polymorph interactions were, therefore, done. Do polymorphs phagocytose virus particles if given the opportunity, and if so are they damaged or can they support the growth of virus? Hanson, Kempf, and Boand (73) showed that influenza virus was phagocytosed by and probably did not grow in mouse polymorphs, but, in much of the published work on the interaction of viruses with leukocytes, the cell types have not been characterized.

The fluorescent-antibody method seemed the only one likely to yield very conclusive results, and therefore ectromelia virus was used. Polymorphs were obtained from the peritoneal cavity of mice 4 hr after the intraperitoneal injection of sterile broth. Peritoneal washings were made in Hank's solution containing 5% normal mouse serum and 5 units of heparin per ml, and introduced into rings (32). Of the cells thus obtained, 75% were polymorphs. Large amounts of the Moscow strain of ectromelia virus were then added, and events were followed by fluorescent-antibody staining. At 2 hr, there had been an extensive uptake of virus by polymorphs; the cytoplasm of many was brightly fluorescent, and others contained no detectable antigen. There had been no significant reduction in polymorph fluorescence by 4 or 8 hr. By 18 hr, most polymorphs were degenerating, and fluorescent amorphous collections of what was presumed to be polymorph cytoplasm lay extracellularly. Nonfluorescent polymorphs were also degenerating. Occasional macrophages had yielded virus and contained fluorescent inclusions, while others had phagocytosed polymorphs.

It was concluded that, given an opportunity, polymorphs phagocytose ectromelia virus quite readily. Perhaps there is some digestive degradation of phagocytosed virus particles, although no fading of antigen was detectable in the experiment. Polymorphs are difficult to keep alive for long in vitro, so that their degeneration was probably not attributable to the action of virus. There was no evidence that they supported the growth of virus.

From the very limited evidence available, therefore, it appears that polymorphs, not being notably involved in the response to virus infection, do not often have an opportunity to phagocytose virus or become infected. When given this opportunity, they may phagocytose viruses, but are not necessarily infected. When they degenerate, they are phagocytosed by macrophages. It is difficult to imagine that they play a very important part in the pathogenesis of virus diseases.

DISCUSSION

The present review deals principally with macrophages, viremia, and lymphoid tissue, three important and neglected aspects of the pathogenesis of virus diseases. Many other important aspects of the subject have been ignored. Thus, there is very little overlap with Bang and Luttrell's (9) recent admirable survey

of the pathogenesis of virus diseases. Suter (150) reviewed the part played by phagocytosis in infectious diseases but did not include virus diseases.

This is not the first time that the macrophage system of cells has been assigned an important role in virus diseases. Brunner et al. (28), for instance, pointed out that macrophages seem to treat virus particles like other colloidal particles, and Ledingham (87, 88) and Beard and Rous (13) drew attention to the importance of reticuloendothelial cells in vaccinia infections of the rabbit. Fenner (55) suggested that in mice infected with ectromelia virus the reticuloendothelial cells in the liver and spleen took up circulating virus, and that this led to the infection of these organs. Florman and Enders (59) pointed out that monocytes, since they move freely through the tissues, and can be infected with vaccinia, could readily spread virus throughout the body. In the present account of the role of macrophages, however, the matter has been dealt with more thoroughly and conclusively, because of the fluorescent-antibody evidence which makes it possible to say exactly which cells are infected. It should be pointed out that nearly all the evidence concerns poxviruses, and the role of macrophages in other virus diseases has in most cases been postulated rather than proven. Because they take up smaller particles less rapidly, macrophages may be less important in infections with smaller viruses.

Macrophages are also of particular importance in a few virus diseases which have not been referred to above. The Warthin-Finkeldy giant cells which figure so prominently in measles are of the macrophage series, and a full understanding of the pathogenesis of this disease will certainly involve discoveries as to the origin and significance of these cells. In this review, most of the evidence for the importance of macrophages concerns mice infected with mousepox, and it is not surprising that macrophages also figure prominently in the lesions of smallpox (27). In rabbits infected with myxomatosis, the myxoma cells themselves may be derived from tissue histiocytes, and Ahlström (1) considered that the pathological changes were sufficient justification for calling this disease an infectious reticuloendotheliosis. In vitro observations on macrophage or mononuclear cell-virus interactions have also been made (13, 18, 49, 60), but as a rule the findings have not been related to events in infected animals, and sometimes there have not been any very firm conclusions as to the type of cell involved (11).

In considering macrophages as comprising a single system of cells, it cannot be taken for granted that macrophages from all parts of the body behave in the same way. Virulent strains of ectromelia virus have been shown to grow in several types of mouse macrophage, including Kupffer cells (107) and histiocytes (127) in vivo, and in peritoneal (Roberts, *unpublished data*; Mims, *unpublished data*), lung (126), and splenic (Mims, *unpublished data*) macrophages both in vitro and in vivo. It is not known, however, whether they are all equally susceptible. Gross differences would seem to be unlikely, and Roberts (*unpublished data*) has shown that virulent and avirulent strains of ectromelia virus differ in the same way both in Kupffer cells in vivo and in peritoneal macrophages in vitro. Bang and Warwick (8) found that macrophages from two strains of mice differed in the same way in their response to mouse hepatitis virus, whether they were from the liver, lung, or heart. Thus, for a given virus, the type of virus-macrophage interaction described in the liver (see above) might also be expected with macrophages in other parts of the body.

No attempt has been made to assess the role of opsonins in the phagocytosis and intracellular fate of virus particles, because in spite of its possible importance no studies have yet been reported. Specific antibodies, certainly, promote the phagocytosis of virus particles (28) and also their intracellular digestion (see above). The phagocytosis of colloidal particles implies a primary attachment of particles to the cell surface, and, while this may be initially electrostatic in nature (6), specific receptor sites may also be involved. The importance of specific cell receptors in the infection of epithelial cells by myxoviruses is established (76), and their role in poliovirus infection has been pointed out (77). Are specific receptor sites involved in the phagocytosis of virus particles by macrophages? In unpublished experiments, it was found that massive intracerebral doses of purified receptor-destroying enzyme injections given to mice 1 hr before toxic intracerebral injections of influenza virus failed to prevent the growth of influenza virus in the cells lining the subarachnoid

spaces (109). The destruction of cell receptors, however, may not have been complete, so that this is no more than suggestive evidence that phagocytosis by macrophages does not require specific receptors. It may, of course, require specific opsonins.

Not much is known about the intracellular events which follow phagocytosis. A limited amount of work has been done on microphages (36, 37, 38, 75), but for macrophages more studies of the type recently reported by Cohn (39, 40) are needed. If interferon is produced by macrophages, as suggested by the work of Gresser (69*a*) and Glasgow and Habel (64*a*), it might play an important part in the response of macrophages to virus infections. After infection there is often, perhaps, an intracellular struggle between the inactivating or digestive powers of the cell and the growth-initiating or enzyme-inducing powers of the virus. A full understanding of these matters must await advances in our knowledge of the eclipse period in virus infections, the production of intracellular enzymes and antiviral substances, and the biochemistry of normal cells.

Much of the published work on macrophages has dealt with their phagocytosis of inert particles and bacteria, and the ability to phagocytose bacteria has been correlated with host resistance to infection. Clearly, this can only be done when greater phagocytic rates are accompanied by greater digestive ability, and, in any case, as was pointed out by Boehme and Dubos (25), such correlations do not necessarily imply a direct causal relation between the phenomena. For viruses, there is less justification for relating phagocytic rates to host susceptibility, although an increased rate of phagocytosis does imply greater host resistance if it is accompanied by an increased intracellular digestive ability. The Moscow strain of ectromelia virus grows well in mouse macrophages, and rapid phagocytosis, if anything, simply hastens the pathogenic process. Significant differences in the rate of uptake could be of importance insofar as other cells, like capillary endothelial cells, had as a result more or less opportunity to be infected. If macrophages take up virus particles very rapidly, they are even more likely to control the course of infection.

Information is needed about the sites of origin, length of life, and movement through the body, of macrophages. Preliminary experiments in which macrophages were labeled with tritiated thymidine (Mims, *unpublished data*) indicate that most mouse peritoneal macrophages live for less than 1 week before dividing or dying, although they may live longer if loaded with India ink. The work of Edwards and Klein (51) and Bertalanffy and Leblond (19) also give some information about production sites and production rates. The findings of Easton (50) suggested that Kupffer cells are mobile and may even move to the lung, and the role of migrating tissue macrophages and monocytes in virus infections has been referred to above. Clearly, more work on these aspects of the macrophage cell system is needed, and will provide a clearer understanding of the behavior of macrophages not only in virus diseases but also in diseases caused by protozoa and intracellular bacteria.

SUMMARY

In this survey of the pathogenesis of virus infections, the usefulness of the fluorescent-antibody technique has been emphasized and certain neglected topics discussed. No attempt has been made to review the subject in a general way. The poxviruses figure prominently because the fluorescent-antibody technique works well for these viruses, and many unpublished observations are reported.

A large section deals with macrophages in virus diseases. Macrophages, in the course of their natural behavior as scavenger cells, phagocytose virus particles. Because they are widely distributed throughout the body and monitor the main body fluids and tissue spaces, they encounter virus particles early in infection. The course of the disease may be profoundly influenced by this virus-macrophage encounter, which in some cases determines the susceptibility of the animal to the disease. All possible types of virus-macrophage interaction have been shown to occur in the liver, and macrophages play an important part in the growth of viruses in this organ.

Most of the experimental evidence implicating macrophages has been obtained from fluorescent-antibody studies of mice infected with ectromelia virus. The role of macrophages in other virus infections has in most cases been postulated rather than proven.

The conditions for the establishment and

maintenance of viremia have to be thought of in relation to the clearance of virus from the blood by macrophages. Viremias are classified into four types.

If viruses which infect capillary endothelium are also cleared from the blood by macrophages, the infection of capillary endothelium will be influenced by the activity of macrophages.

Experiments on the growth of ectromelia virus in spleen and lymph nodes are described, and an attempt is made to assess the part played by lymphoid tissue in virus infections. The immune response is discussed with particular reference to the occurrence in lymphoid tissue of macrophages in intimate relation to lymphoid cells.

Short accounts are given of virus infections of certain other organs and tissues.

The discussion is devoted to a further consideration of virus-macrophage interactions.

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