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ANIMAL VIRUSES OF ECONOMIC IMPORTANCE: GENETIC VARIATION, PERSISTENCE, AND PROSPECTS FOR THEIR CONTROL

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1. INTRODUCTION

This article is intended as a review of the relevant properties and methods of control of those viruses which are economically important to humans. The viruses included are those which directly affect livestock and other important animals such as fishes, and those which are transmitted from animals to humans with serious consequences. Other viruses have been included in the discussion however, where they serve as better examples to illustrate concepts.

Two major themes are emphasized. The first concerns the genetic changes that take place in the viruses and the resulting implications for viral pathogenesis, epidemiology and diagnosis. Viruses do not necessarily remain genetically stable during the course of persistent infection or during their spread from one population of animals to another. In fact recent molecular analysis has revealed a remarkably high mutation frequency, especially in RNA genomes. The second theme concerns the methods of controlling virus infection. The latter topic includes discussion of current control practices and their limitations, and also discussion of newer methods based on recent technological innovations, such as the application of recombinant DNA techniques. These two themes are not unrelated however, and future success in virus control will only be achieved by taking into account those aspects of viruses which have recently begun to receive their due recognition, i.e. properties such as changes in virulence, persistence within the host, and others.

This review is not intended to serve as a source of information on animal viruses and their infections. Rather an attempt has been made to discuss principles and concepts, with reference to specific examples for illustration. Additional specific details can be found in books on veterinary virology, the most recent of which are those by Mohanty and Dutta (1981) and Gibbs (1981). It should be emphasized however, that many of these concepts have traditionally received scant attention in textbooks.

The subject of classification of animal viruses into discrete families is under continuous discussion and revision, both from the philosophical and practical standpoints. Table 1 summarizes the current situation, which provides for some 17 families containing viruses relevant to this review (Matthews, 1981; Melnick, 1982). The examples cited represent those referred to in the text. An interesting review of the subject of virus taxonomy was recently published (Matthews, 1983).

Viral taxonomy is a difficult subject, which probably occupies more of our time than it really should. It is an unfortunate fact that scientists in general have the urge to categorize all organisms into neat little pigeon holes, based upon whatever features of the organisms happen to be popular at the time. Although this undoubtedly aids in making reference to individual viruses, it also results in the necessity of frequent alterations in the taxonomic scheme, to take into account the emergence of newly discovered features.

TABLE 1. *Classification of Animal Viruses*

Virus family	Pictogram	Dimensions (approx. nm)	Type and size of genome*	Replication class (1–7) (see Fig. 4)	Individual members referred to in text†
Iridoviruses		$d \geqslant 130$ varies	ds DNA $\geqslant$200,000 np	1	*African swine fever* (ASFV) Lymphocystis (LCV) piscine erythrocytic necrosis (PEN) viral erythrocytic necrosis (VEN)
Pox viruses		~300 × 200	ds DNA ~200,000 np	1	*vaccinia* smallpox various mammalian pox viruses
Herpes viruses		$d \sim 180$	ds DNA 130,000–240,000 np	1	*herpes simplex* 1, 2 (HSV-1, 2) *cytomegaloviruses* (CMVs) *Epstein–Barr* (EBV) varicella-zoster (VZV) *Marek's disease* (MDV) *murine cytomegalovirus* (MCMV) equine abortion (EAV; EHV-1) *pseudorabies* (Aujesky disease) (PRV) equine herpes, 2, 3 infectious bovine rhinotracheitis (IBRV; BHV-1) bovine herpes 2, 3, 4 (BHV-2,3,4) rat CMV guinea-pig CMV pig CMV infectious Laryngotracheitis (ILV) turkey herpes (THV) channel catfish (CCV) herpes virus salmonis (HVS) turbot herpes walleye herpes Pacific cod herpes
Adenoviruses		$d \sim 80$	ds DNA	1	human adenoviruses (types 1–40 etc.) animal adenoviruses
Papovaviruses		d 40–55	ds DNA ~5–8000 np	1	papillomaviruses bovine papilloma-1 (BPV-1) simian virus 40 (SV40) polyoma JC virus
Parvoviruses		$d \sim 20$	ss DNA ~5000 n	2	parvovirus present in many animal species
Hepadnaviruses		d 45	ds DNA ~5000 np	7	hepatitis B (HBV) similar viruses in ducks, rodents
Retroviruses		$d \sim 100$	ss RNA (diploid) ~10,000 n	6	Rous sarcoma (RSV) avian leukosis-leukemia Friend leukemia (FLV) *bovine leukemia* (BLV) human T-cell leukemia (HTLV) visna maedia progressive pleuropneumonia of sheep
Coronaviruses		$d \sim 100$	ss RNA ~27,000 n	4	murine hepatitis

TABLE 1. (cont.)

Rhabdoviruses		180 × 70	ss RNA ~12,000 n	5	*vesicular stomatitis* (VSV) *rabies* bovine ephemeral fever (BEFV) *infectious hematopoietic necrosis* (IHNV) viral hemorrhagic septicemia; Egtved (VHS) spring viremia of carp (SVC) swim bladder inflammation (SBI) pike fry rhabdovirus (PFRV) Rio grand perch
Arenaviruses		*d* ~ 120	ss RNA (2 segments) ~15,000 n	5	*lymphocytic choriomeningitis* (LCMV) Lassa fever
Bunyaviruses		*d* ~ 100	ss RNA (3 segments) ~18,000 n	5	Hantaan Bunyamvera viruses Nairobi sheep disease
Reoviruses		*d* ~ 70	ds RNA (10–11 segments) 20–25,000 np	3	reoviruses 1–3 *rotaviruses* *bluetongue* (BTV) African horsesickness
Paramyxoviruses		*d* > 200	ss RNA ~20,000 n	5	*rinderpest* (RPV) bovine parainfluenza-3 *Newcastle disease* (NDV) *Measles* Simian virus 5 (SV5) Canine distemper (CDV)
Orthomyxoviruses		*d* ~ 100	ss RNA (8 segments) ~17,000 n	5	*influenza A, B, C* fowl plague (FPV) swine influenza equine influenza
Togaviruses		*d* 40–70	ss RNA ~13,000 n	4	*Semliki* forest (SVF) *Sindbis* (SV) hog cholera (HCV) bovine viral diarrhea (BVD) *dengue* yellow fever equine encephalitis viruses Rift Valley fever rubella
Caliciviruses		*d* 35	ss RNA ~8000 n	4	Vesicular exanthema San Miguel sea lion virus type 5
Picornaviruses		*d* 25–30	ss RNA ~8000 n	4	*foot-and-mouth-disease* (FMDV) polioviruses hepatitis A swine vesicular disease Coxsackie B
[Birnaviruses]‡		*d* 60	ds RNA (2 segments) ~7000 np	3	*infectious pancreatic necrosis* (IPNV) *infectious bursal disease* (IBDV)

Legend:
*d, diameter; ss, single stranded; ds, double stranded. Size is given in nucleotides (n) or nucleotide pairs (np).
†Viruses italicized are discussed in some detail in the text.
‡Not definitely assigned family status.

The historical basis for the subdivision into families was virion morphology, as determined by electron microscopy. Subdivision has since been popularized on the basis of antigenic relatedness, replication schemes and, more recently, genetic relatedness (Melnick, 1982; Matthews, 1983). The nature of the classification attempt is a reflection

of the relative sophistication of the technology available, and no doubt in a few years we shall be able to compare all the viral genome nucleotide sequences. Unfortunately viruses do not fall into convenient groups of features, with the possible exception of gross morphology. Instead they are continuously undergoing genetic change, with consequent changes in their protein composition. The logical extension of current viral taxonomy is the development of a separate category for each individual virus ever isolated.

The problems inherent in viral taxonomy are compounded by the lack of consistent correlation between virus type (family, genus etc.) and disease. Viruses from unrelated families may cause very similar disease patterns, e.g. hepatitis A (an enterovirus) and hepatitis B (a hepadnavirus) in humans; or the several viruses which cause clinically similar swine vesicular diseases, viz. porcine enterovirus type 9; foot-and-mouth-disease virus (a different picornavirus); vesicular stomatitis virus (a rhabdovirus); and vesicular exanthema virus (a calicivirus). On the other hand individual viruses within a given family may cause totally different forms of disease, e.g. herpes simplex virus; Epstein–Barr virus and cytomegalovirus, all of which are human herpes viruses and which produce different patterns of disease.

The nomenclature of viruses has suffered even more confusion and controversy. Generally Virologists talk about viruses in understandable colloquial terms; but the 'proper' or 'official' names may be in various cases English, Latin, Greek or other language, or a combination of them. Often, though not always, the names reflect the disease. It is evident that no universally acceptable nomenclature has been proposed. Consequently in this review viruses will continue to be referred to by their traditional names, with some degree of generic qualification, when necessary. Table 1 has therefore been constructed in order to allow easy identification of the viruses mentioned in the text.

1.1. Economic Aspects of Virus Diseases

It should be self-evident that viral disease in livestock can have a profound economic effect on humans. To the producers and merchants it means a decrease in livelihood, either because of the loss in marketable animal products, or because of a loss of performance in the case of draught or recreational animals. To the ultimate consumer it means increased price and decreased availability.

The impact of dead and diseased animals is obvious in this respect. But in addition animals afflicted with sublethal or chronic infections may also become uneconomical due to such things as slow weight gain, low yields of milk or eggs, suboptimal quality of the final product.

In order to put the problem into perspective it is useful to consider some facts and figures. Table 2 lists the current major livestock in different regions of the world, together with the human populations according to estimates available to 1981 (Animal Health Yearbook, 1981). Regional disparities in the distribution of species are of course hidden in this simplified scheme. For example, Australia and New Zealand rely heavily on an abnormally high population of sheep, respectively 9 and 23 per capita, and of cattle, with corresponding values of 1.7 and 2.7 per capita. Their animal products are largely exported, thus a high standard of veterinary health is essential to these countries.

Table 2. *Regional Distribution of Principal Livestock (in Millions)**

Region	Cattle	Horses	Sheep + goats	Pigs	Poultry	Humans
Americas (N, C and S)	390	32	152	147	1400	636
Europe + Middle East	160	2	290	173	1530	620
USSR	115	<1	148	74	1300	265
China	53	11	187	305	820	940
Asia (excl. China) + Australasia	315	6	453	63	1270	1500
Africa	156	3	318	8	610	463
Totals	1190	55	1548	770	6930	4424

*Source of data: Animal Health Yearbook, 1981.

Some general conclusions can however be drawn from the figures in Table 2. In terms of sheer numbers poultry are the most frequent livestock (avians out-numbering humans by 50%), followed by sheep plus goats, cattle, pigs, horses and others. Pigs are generally unimportant in African countries, while horses are particularly important in some Asian and American countries. The relative numbers will probably change however, in accordance with changes in local economic factors, the depletion or acquisition of grazing land, and husbandry practices.

It is difficult to ascribe meaningful monetary values to livestock since their value varies tremendously from one region to another. Nevertheless in 1980 total US stocks of cattle, pigs, sheep and horses were estimated at $90 billion. But in general cattle on a global scale assume greatest economic importance, followed by sheep and pigs.

As an illustration of an in-depth economic study of viral disease on a community, it is well worthwhile examining the US study on the potential impact of foot-and-mouth-disease (FMD) in that country. The report compiled and summarized the results of a simulated epidemic of FMD virus followed by the implementation of various alternative control programs (McCauley *et al.*, 1979). This virus is not endemic in the USA and is normally restricted by selective importation of animal products. Estimates were made of the current annual consumption in North America of those foods directly affected by a FMD virus epidemic. Losses arising from such an epidemic, due to financial losses combined with increased consumer costs, were estimated to be 12 billion dollars (1982 value). Cost benefit analysis showed clearly that the present policy of restrictive entry was considerably more beneficial than alternative schemes which would allow the virus to become endemic and accompanied by various vaccination schemes.

In countries where the virus is endemic, efficient and complete vaccination is necessary to restrict its spread. The consequences of uncontrolled global spread of this one virus are difficult to comprehend. As many of the developing countries turn to the 'western-style' methods of raising livestock it becomes increasingly important to ensure continuous vigilance against current and future potential epidemics of viral disease.

Fish were excluded from Table 2 because of the difficulty in obtaining actual numbers. Fish are of great economic importance and may in the future comprise a higher proportion of our edible protein.

Estimates for global farmed fish in 1975 were approximately 4×10^6 tonnes, of which more than half were represented by carp in China (Hill, 1981). Another recent estimate quotes a value of 5.7×10^6 tonnes of aquaculture-derived fish for 1978, 70% of which came from South and South-East Asia (Davy and Graham, 1979). These figures compared with 70×10^6 tonnes of wild caught fish. One would expect these values to move toward each other in the near future as wild species are depleted and fish farming becomes more popular.

Increased demands however will probably be met by farming practices of selected species, although increased artificial rearing of fish will undoubtedly result in higher frequency of viral disease and consequently better surveillance and control programs will be needed.

At present several viruses cause tremendous losses to hatcheries, e.g. viral hemorrhagic scepticemia (VHS) in European countries, infectious hematopoietic necrosis virus (IHNV) in Alaska and in the Columbia river basin area. A recent report on the latter area indicated that in 1981 the cumulative IHNV-caused loss of trout and solmonid eggs and juveniles from government operated hatcheries in this area was 7.7×10^6. The corresponding value for 1982 was 6.6×10^6 (Groberg, 1983). This amounted to as much as 70% of the stocks in some hatcheries. The incidence of viral disease appears to be much less in 'sea-ranching' and 'pen' operations.

The discussion so far has focused on livestock animals as food producers. In addition consideration should also be given to the economic impact of viral disease on game animals. For example hunting and sport fishing are important 'industries' in many countries; as is horse racing and to a lesser extent other animal sports. Game 'watching' is an important source of tourist dollars in some African countries.

Rabies virus is a continuous threat to livestock in many areas. A group of South American countries recently estimated their combined losses due to rabies in excess of 28 million dollars (US) (Callis *et al.*, 1981).

Rinderpest, though now controllable, once ravaged Europe, where it has been estimated that in the 18th century over 200 million cattle were lost to this virus.

However, even controllable diseases are not so easy to eradicate, partly because of the tendancy of viruses to change (see Section 4) and persist in carrier populations, and partly for other reasons, e.g. in India, where slaughter of diseased cattle is antireligious, and consequently foci of endemic FMD and other infections remain.

In view of these facts and considerations it seems prudent to examine, and to be aware of, the prospects and limitations for control of animal viruses.

2. TRANSMISSION OF VIRUSES

In order to succeed in evolutionary terms, viruses must be capable of replication, persistence within a host, and transmission to other individual hosts of the same or different species. Some viruses are strictly species specific, while others have adapted themselves to many different hosts. Since viruses are generally unstable in the environment, transmission in the wild is usually limited to members within a herd, except when direct contact is made between animals of different species. The latter may be effected during fighting, by sharing water-holes, or by the consumption of virus-infected fluids and tissues by predators. However, many viruses have circumvented this limitation by making use of insect vectors, especially mosquitoes and ticks. There are more than 400 so-called 'arboviruses' (ARthropod BOrne viruses), representing several different virus families, which have adapted to growth in the cold blooded invertebrate host as well as many vertebrate species (McLean, 1980). Such viruses are capable of transgressing species barriers readily. Two examples are depicted in Fig. 1.

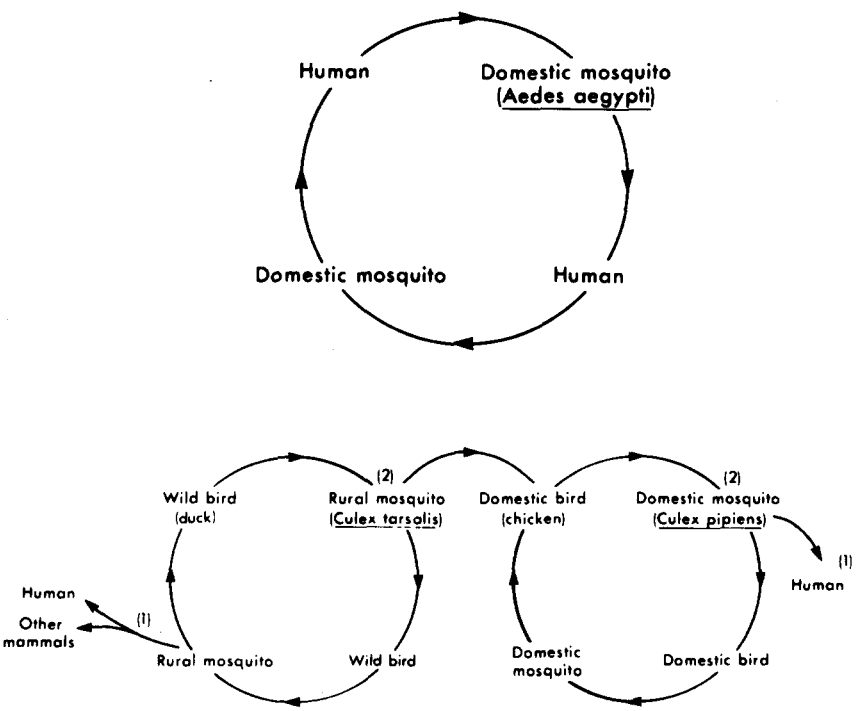

FIG. 1. Examples of vertebrate–arthropod cycles of virus infection. The upper diagram represents a relatively simple cycle involving human, mosquito and togavirus such as dengue virus. The lower diagram illustrates a more complicated cycle involving bird–mosquito interactions, and mammals (e.g. horses, humans) as 'dead-end' hosts. An example is western equine encephalitis (WEE) virus. Reprinted from McLean (1980) with permission.

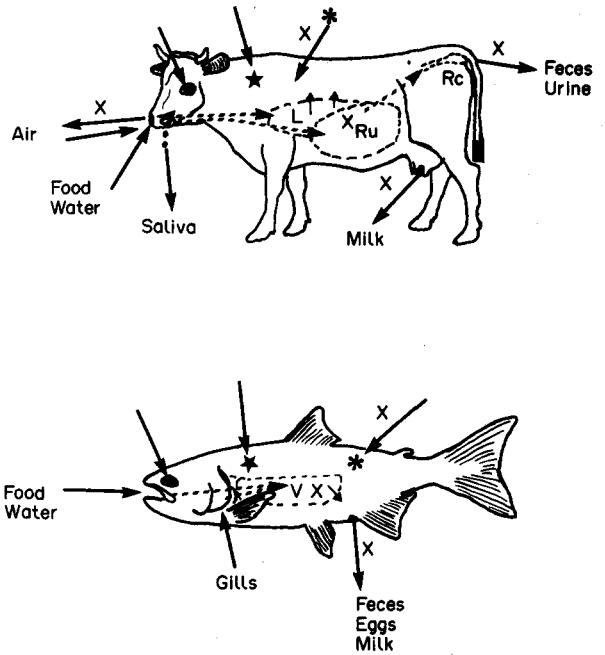

FIG. 2. Routes of transmission of viruses in mammals (upper drawing) and fish (lower drawing). Arrows indicate routes of transmission; ×, points of possible control; ★, surface wounds; ✱, vectors (arthropods, ectoparasites); L, lungs; Ru, rumen; Rc, rectum; and V, viscera.

In contrast, domesticated animals of different species are often deliberately maintained in close contact, thus permitting viruses more opportunity of cross-infection. Since the intervention of humans and their traditional husbandry practices, viruses have probably encountered more opportunities for adapting to novel hosts.

In general these same principles apply also to the aquatic environment, with some differences. The environment itself is inherently less hostile towards virus survival. Therefore direct transmission between fish is probably frequent. In addition aquatic animals may become infected by eating infected prey. Less is known about vector transmission than it is for terrestrial animals, although the opportunities are certainly there, in view of the abundance of parasites.

2.1. TRANSMISSION WITHIN A SPECIES—HORIZONTAL

This refers to non-vector mediated transmission from an exogenous source, or the acquisition of a placentally transmitted virus by a developing fetus. An infected animal may excrete virus from the respiratory passages in the form of aerosols or droplets, or in various secretions and excretions such as saliva, urine, feces, milk, semen and ovarian fluids. Recipient animals may become infected by consuming contaminated food and water, or by feeding on infected carcasses, or by contact with the excretions mentioned above. In the case of fish viruses, the water itself can serve as an excellent medium for transmission (see Fig. 2). The skin, with its various coverings of hair, wool, scales etc., should provide a formidable barrier to direct virus infection, except that the skin of wild and domestic animals is frequently broken by wounds, abrasions, ulcers etc., which could permit easy access to viruses. Pox virus and papilloma virus lesions are thought to arise principally from blood borne virus. However, the skin can also be a source of infectious virus, e.g. Mareks disease virus of chickens, which is transmitted by the feather follicles (Payne, 1982).

Successful transmission of virus from one animal to another is no guarantee of a successful infection in the new host. In many cases a certain threshold level of infectious virus particles is required and this level is quite different for different viruses. Thus, the excretions must be able to produce sufficient virus in order to attain this threshold. Furthermore the threshold level may depend upon the physiological status of the recipient,

and different species of recipients undoubtedly differ with respect to threshold levels required.

Many viruses need a phase of viremia (i.e. blood borne virus) in order to establish an infection. Blood and lymph serve as convenient vehicles for ensuring dissemination to all or many tissues. What happens after the initial virus–tissue encounter is dependent upon a given virus–tissue combination, and is difficult to predict in advance, since many biochemical events may follow. These are considered in more detail in Section 3. Incompatibility between virus and cells at any time in the early stages of infection may prevent the virus from establishing a good infection.

To some degree the transmission of virus from one animal to another is amenable to control. Thus measures such as avoidance of too close contact, separation of food and water supplies, and isolation of demonstrably infected animals, will all tend to reduce the incidence of many virus infections. In human populations it has been shown that the transmission of enteric viruses such as poliovirus can be dramatically reduced by the application of good hygienic practices.

Vector mediated transmission is at least theoretically preventable (see Section 9), but the direct spread of virus through the air or water is probably not (see Fig. 2).

One of the classic examples of a virus which spreads readily through the environment is the foot-and-mouth disease virus (FMDV). The virus infects cattle, sheep, goats and pigs with serious consequences, especially in young animals, although horses are spared. Other animals, including humans, may also be infected. Various excretions and secretions contain high concentrations of infectious particles, which may be transmitted in droplets over kilometers on prevailing winds. The virus can also be conveyed on clothing and in stored hides and meat products (Pereira, 1981).

The success that this virus has achieved in transmissibility and infectivity, and its consequent economic importance, has long aroused an interest in control measures. In addition to the appropriate sanitary and quarantine measures which are applied to prevent widespread epizootics, vaccination programs have always been of great concern. These are discussed in more detail in Section 9, while other pertinent properties of FMDV are considered in Sections 4 and 10.

Several other viruses, which clinically resemble FMD, are also transmitted through the environment, but are much more host specific. Examples are hog cholera virus (HCV), also known as swine fever virus; vesicular exanthema virus (VEV); and swine vesicular disease virus (SVDV) (Mohanty and Dutta, 1981). These all belong to different families (Table 1).

Many other viruses however are more restricted in their mode of spread. Thus excretion may be limited to respiratory fluids, where viruses are conveyed in droplets or aerosols, or to feces. Many of the myxoviruses are transmitted by the former route, and these may be species specific, for example bovine parainfluenza 3 (PI3), or relatively non-specific such as rinderpest (Mohanty and Dutta, 1981). Examples of fecally transmitted viruses are the many rotaviruses, most of which are species specific (see Section 10.9).

A number of viruses are known to be capable of crossing the placenta of vertebrates. Notable examples are the togaviruses, bovine virus diarrhea (BVDV) and hog cholera virus, and some herpes viruses, e.g. equine abortion virus (Mohanty and Dutty, 1981). This method of transmission is not always of benefit to the virus however, since it often results in fetal death or abortion. But in egg-laying animals, this can represent an important method of transmission, as exemplified by several fish viruses (Wolf, 1972; Pilcher and Fryer, 1980a,b). Thus the infectious pancreatic necrosis virus (IPNV) and the fish rhabdoviruses are often found as external contaminants of eggs, which have often been implicated as carriers of these viruses to new aquatic environments (usually by courtesy of human intervention).

Saliva is a common source of virus for transmission to other members of the same species within close contact. This is especially true for some viruses, notably the cytomegaloviruses, which persist in salivary glands long after the primary infection. Mothers who secrete virus in their saliva can readily transmit these viruses to suckling

infants who may not be immunologically competent to prevent infection (Hudson, 1979, 1984). Thus from the point of view of the virus, saliva would seem to offer an ideal means of ensuring continual transmission to the offspring.

2.2. Transmission Within a Species—Vertical

This mode of transmission implies transfer of the virus from parent through the germ-line cells. Many members of the retroviridae accomplish this by integrating proviral DNA sequences into the host DNA (Mims, 1981). Examples are the avian leukosis and leukemia viruses, e.g. Rous sarcoma virus (RSV), and probably bovine leukemia virus (BLV) (Kaaden and Lange, 1984). The significance of BLV or other vertical virus transmission has assumed greater concern recently because of the risk of introducing the virus into new herds as a result of embryo transplants or allogeneic sperm (Eaglesome *et al.*, 1980; Kahrs *et al.*, 1980; Bowen *et al.*, 1983). Conceivably the recipient strain of the animal might not be able to 'control' the virus as stringently as the donor strain, and consequently the new virus may no longer remain latent but instead may proliferate, accompanied by serious damage in the fetus or newborn. Crossing strain barriers in mice has frequently led to the emergence of previously unrecognized retroviruses (Phillips *et al.*, 1977; Rapp and Todaro, 1978).

In theory any other virus with the capacity to integrate viral DNA into host DNA, such as some papovaviruses, could be transmitted to the offspring in this manner. Integration may not be essential however, since there have been claims of true vertical transfer of other kinds of virus from mothers to offspring, although it is always difficult to completely rule out the possibility of virus binding adventitiously to ova. Such is the case with fish viruses, as noted above. There does appear to be an exception however in the case of infectious pancreatic necrosis virus in zebra fish, where egg-mediated transmission to the progeny was achieved, and the transmitted virus persisted for at least several months (Seeley *et al.*, 1977).

2.3. Transmission Between Species

Viruses which are spread through the environment in large amounts are likely to infect animals of different species. Foot-and-mouth disease virus (FMDV), referred to already, is an excellent example. The major obstacle to indefinite spread between species is the genetic resistance barrier. Thus horses are resistant to FMDV, presumably because the virus is unable to replicate in the equine cells initially encountered by the virus.

Many viruses are spread by the respiratory route and could conceivably infect other species which come into the vicinity of an infected animal. Newcastle disease virus (NDV) spreads rapidly amongst chickens when introduced into a flock, and can also infect other animals in close contact (Lancaster, 1981).

Oral–fecal spread is probably less common between species, unless food and water are shared. However this method has been implicated in transmission of influenza A by migratory birds to ponds and lakes frequented by vertebrate wildlife (Hinshaw *et al.*, 1978; Laver and Webster, 1979). Similarly some birds may carry infectious pancreatic necrosis virus (IPNV) from one aquatic habitat to another (Sonstegard and McDermott, 1972).

Salivary secretions are less important modes of transmission across species barriers, except when fighting takes place between individuals. Rabies virus may be thought of as an exception, although most animal species which become infected die as a result of the infection. Consequently the infection tends to be preserved within selected carrier species such as bats, foxes or skunks, depending upon the locality (Crick, 1981).

A very effective mode of transmission is by consumption of the flesh of infected animals. Viruses are generally sufficiently stable in fresh tissues and body fluids to allow this to happen. Furthermore the conditions leading up to death, e.g. the stress of a long chase by a predator, or the stress of spawning in Pacific salmon, may be enough to reactivate a persistent virus infection, with the result that relatively large amounts of virus are present in the carcass. In the case of the spawned out salmon such viruses will be available to infect

not only the eggs; but also any aquatic form of life in the vicinity of the carcass, as well as the birds or terrestrial wildlife feeding on the carcass.

2.4. TRANSMISSION BY VECTORS

Many viruses are transmitted from one vertebrate animal to another by means of an animal vector. The vectors are usually mosquitos and ticks, although several other arthropods such as sandflies and midges can serve this function. In addition, transmission can be mediated by parasites and by vertebrates. The viruses concerned include most togaviruses; various members of the families bunyaviridae, rhabdoviridae; the genus orbivirus; and a few additional viruses from other families (McLean, 1980). The documented incidence of non-arthropod transmissions will probably increase as investigators become more aware of the importance of other vectors.

The arthropod borne infections can be regarded as cycles in which warm-blooded vertebrates alternate hosts with the cold-blooded arthropod (Fig. 1). The viruses concerned have evidently become versatile and adapted themselves to growth in these different hosts. The mosquito or tick obtains virus from the blood of the vertebrate and then has to amplify the level of virus in order to pass on in its saliva sufficient virus to establish an infection in the second vertebrate host. The natural vertebrate host is very often a small mammal such as a rodent. In addition viruses may be passed on from one generation to another within the arthropod itself. Many cases of transovarial transmission have been documented, although it is still a controversial matter whether the virus is passed on vertically or adventitiously.

Humans and domestic animals may participate in cycles of infection either as important components or as accidental dead-end hosts. For example humans can be part of the cycle with mosquitoes in maintaining dengue and urban yellow fever. In contrast the so called equine encephalitis viruses (eastern, western, Venezuelan encephalitis viruses), which can bring about substantial losses to horse breeders in many parts of the American continent, are normally maintained in nature by mosquito–bird cycles. In such cases horses and humans represent dead-end hosts (Fig. 1). Among the mosquito-transmitted viruses of veterinary importance are Rift Valley fever virus (RVFV), which is widespread in Africa and produces epizootics in cattle, sheep and goats. There are many important tick-borne viruses, e.g. Nairobi sheep disease virus, in which sheep or goats form a natural cycle with ticks. In addition there are many so-called tick-borne encephalitis viruses which can infect humans and domestic animals.

Several viruses of economic importance can be transmitted by other insect vectors, although the importance of the vector has been questioned in relation to natural infections, and in some instances even the identity of the vector is questionable. A case in point is African Swine Fever virus (ASFV). This virus originated in Africa but has since been imported into other countries. It can be transmitted between pigs by means of a specific tick vector, or by a porcine louse, or by direct contact without vector intervention (Wilkinson, 1981). Thus the virus has adapted itself to allow for several modes of transmission, although the relative importance of each is unknown. Vesicular stomatitis virus (VSV) can also be transmitted between various species of livestock and wild animals by physical contact, by mosquitoes, and by sandflies. Again it is not known how important each route is. Bovine ephemeral fever virus (BEFV) apparently requires vector mediated transmission, although in this case mosquitos, sandflies and even gnats have all been implicated as vectors. Bluetongue virus (BTV), which infects primarily sheep and goats, can be transmitted by *Culicoides* midges, and in this case the host specificity is determined by the vector (Sellers, 1981).

More attention has recently focused on rodents which, in addition to their participation in arthropod vector–mammal cycles, can also transmit other viruses to humans and presumably to domestic animals. Several viruses of the arenaviridae, including the well-publicized Lassa fever virus, fall into this category. These viruses are apparently quite innocuous in their natural rodent hosts, but give rise to hemorrhagic fevers in humans.

Transmission is thought to occur by contact with contaminated rodent urine or other excretions.

Mice and rats in many parts of the world are now known to serve as carriers for Hantaan virus (a bunyavirus) which, together with related viruses, gives rise to hemorrhagic fever with renal syndrome (HFRS) in humans coming in contact with the infected rodents. Transmission appears to be by contact with contaminated saliva or feces (Peters and Johnson, 1984).

It is quite possible that rodents, as well as other urban and rural animals, act as reservoirs for many other viruses capable of inflicting disease upon humans and their economically important animals. This may become a serious problem as more of the world's virgin territory is turned over to livestock.

Birds, as transmitters of viruses, have begun to receive more recognition recently. Their role as carriers of influenza A viruses has been alluded to already (Hinshaw *et al.*, 1978; Laver and Webster, 1979), as has their potential role in the passive transfer of fish viruses. Birds are attractive vectors from the point of view of viruses because of the great distances they can cover and their tendency to mingle with other animal species. In this regard it is not necessary for the birds to propagate the virus, for some viruses can retain respectable infectivity titers after passing right through the gut (Sonstegard and McDermott, 1972).

The role of parasites as vectors of animal viruses has not received much attention, although the opportunities would seem to be good, especially in the aquatic environment. One very intriguing example was brought to light recently however. This involves a sea lion calicivirus (unfortunately referred to as the San Miguel sea lion virus type 5). The virus can be transmitted to the sea lions by feeding them virus-infected fish of a certain species, or by infecting these same fish with a virus-infected lungworm parasite of the fish. As a result of their investigations, the authors concluded that the virus was part of a parasite–fish–sea lion cycle (Smith *et al.*, 1980). Furthermore they implied that parasites may likewise act as vectors of other viruses, e.g. the pig calicivirus, vesicular exanthema virus.

In view of these recent examples, it is likely that the subject of non-arthropod vector mediated virus infections will receive more attention in the near future.

Figure 2 illustrates diagrammatically the various routes of virus transmission and the possible points of control. The latter will be discussed in more detail in Section 9.

3. REPLICATION OF ANIMAL VIRUSES: TARGETS FOR CONTROL

A virus is essentially a collection of genes enclosed within a protein coat and often, in about half of the known animal viruses, is surrounded by a membrane. Many of these viruses are sufficiently stable in the environment, or in vectors, to ensure their transmissibility to other animals. Intervention in transmission however is difficult to perform in practice. For this reason, and in view of the spectacular successes of antibiotics in controlling replication of bacteria, much attention has focused on methods of interfering with virus replication. Unfortunately there are two basic problems inherent in this approach. Firstly, viruses comprise the same basic chemical makeup as the host cells themselves, and secondly the overall biochemical processes involved in their replication are very similar to analogous host cell processes. In order to circumvent these limitations it is necessary to find ways of either augmenting the body's natural defense mechanisms, or searching for possible unique aspects in the viral replication schemes.

Figure 3 summarizes the common stages in virus replication, and Fig. 4 summarizes the virus replication schemes determined to date. In spite of the thousands of animal viruses identified so far, they fit conveniently into 17 families and, more importantly, into only seven replication schemes. The classification is essentially a modification of the one proposed by Baltimore and is based upon the nature of the viral genome and its requirements for replication (Baltimore, 1971; Bachrach, 1978; Carasco and Smith, 1984; Kucera and Myrvik, 1985). According to this mode of classification, other features such

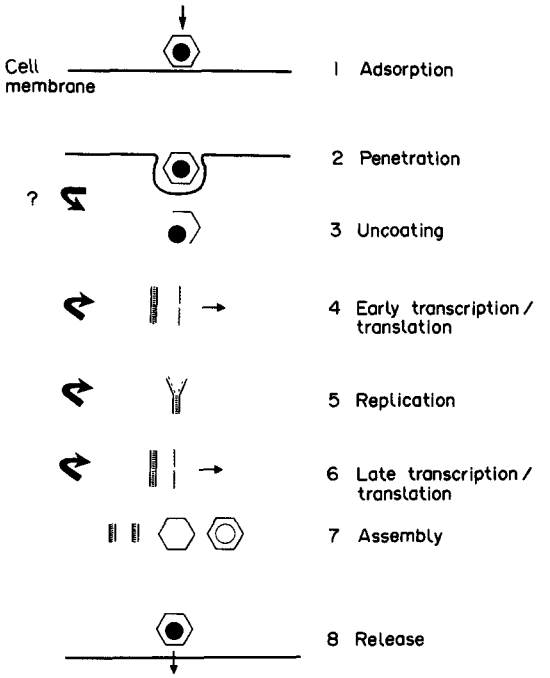

FIG. 3. Stages in virus replication. Thick curved arrows on left side represent possible points of control by antiviral chemicals (details in Section 9).

as mechanisms of virus penetration; uncoating (i.e. removal of extraneous proteins); intracellular site of replication; and details of the packaging and maturation of the viruses (see Fig. 3), are of secondary importance, and seem at present to be less amenable to intervention. Although the specific processes of transcription, translation and DNA replication are essentially the same as the analogous host cell processes, there may be

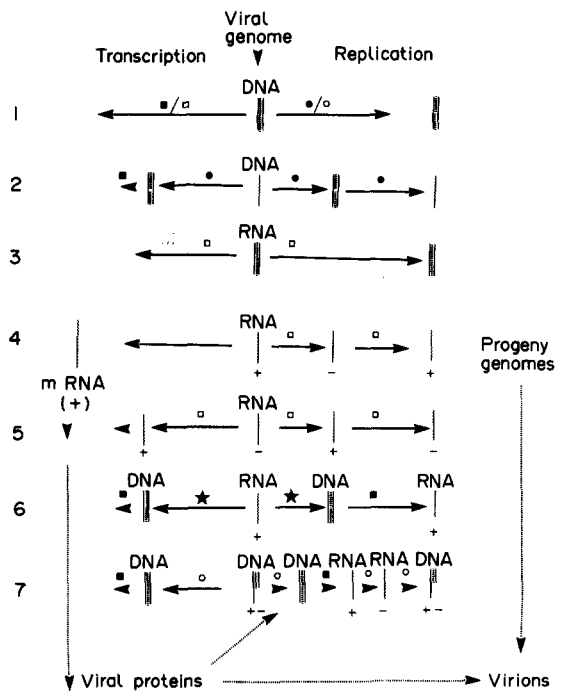

FIG. 4. Schemes of virus replication. For each category (Scheme 1–7), the viral genome is depicted in the middle of the diagram, with transcription proceeding to the left and replication to the right. Symbols: +, positive (messenger) strand of RNA (DNA); −, negative (antimessenger) strand of RNA (DNA); ■, host RNA polymerase; □, viral coded RNA polymerase; ★, reverse transcriptase; ●, host DNA polymerase; and ○, viral DNA polymerase.

sufficient differences in certain details to allow for the design of appropriate chemical inhibitors. The object is of course to find a chemical which can enter the virus-infected cells and block a step in the replication scheme without inhibiting any host cell directed process.

Fortunately many viruses code for specific enzymes, and some of these are sufficiently different from corresponding host cell enzymes to encourage optimism in this approach. Further details will be discussed in Section 9. In the present context, attention is drawn to the so-called 'targets' for antiviral therapy indicated in Fig. 3.

In each of the seven schemes depicted in Fig. 4, the viral genome is centrally located, while stages in transcription and translation are depicted to the left and replication of the genome to the right. Symbols denote the requirements for cellular and viral enzymes. The latter may be packaged within the virions or alternatively may be synthesized in the infected cell. As the number of identified virus-specific enzymes grows, so does the number of potential targets.

It should be realized that the schemes shown may require further elaboration as more details are worked out in the future. By way of illustration, at the time of initiation of this review, six replication schemes were accommodated. Since then however it has become clear that hepatitis B virus and its relatives found in woodchucks, ground squirrels and Peking ducks, i.e. the hepadnaviruses, utilize elements of both DNA-virus and retrovirus replication schemes, and hence must be considered as a separate class (Summers and Mason, 1982).

Thus for the time being we have seven categories. Conceivably a few more may be added when more is known about some of the exotic viruses, but hopefully we shall be spared the necessity of subdividing these into scores of genus- and species-specific replication schemes.

4. PATHOGENESIS AND THE VARIATION OF VIRUSES

This section discusses the factors affecting pathogenesis and the related subject of how viruses vary. The factors may be conveniently divided into: (i) host; (ii) viral; and (iii) environmental. Conceivably it may become possible to modulate some of these factors so as to augment the capacity of the host to resist the establishment of persistence of virus infections.

4.1. Host Factors Affecting Pathogenesis

These may be subdivided into: (i) sites of infection and physical barriers; (ii) host genetics, which includes cellular barriers to virus infection, and determinants of immune responses to viruses; (iii) age of host; and (iv) physiological factors and stress.

4.1.1. *Sites of Infection and Physical Barriers*

It has long been known that certain viruses, such as foot-and-mouth-disease virus (FMDV), can establish infection in many different tissues of susceptible animals, whereas others infect specific 'target' tissues. For example some respiratory viruses enter into a 'cycle' involving nasopharyngeal tissues of the donor and recipient animals, with transmission being mediated by droplets or aerosols discharged from and impinging on these particular tissues. On the other hand different species of animals may react in a different manner to such a virus.

It is often difficult to determine whether these differences are due to anatomical (physical) barriers or to inherent genetic susceptibility or resistance at the tissue level. It should not be forgotten that apparent tissue localization may only reflect the natural mode of transmission of virus. Thus so-called respiratory and genital strains of herpes viruses (e.g. infectious bovine rhinotracheitis virus, IBRV) usually represent only this, rather than the existence of two distinct viruses with two different target tissues (Misra *et al.*, 1983; Ludwig, 1984). Once a virus has entered the blood or lymph it can gain access to many more tissues. For this reason viruses transmitted by biting arthropods easily disseminate

through the body. In contrast the degree of penetration of respiratory viruses is to a large extent determined by the physical properties of the transported particles. Viruses transmitted in smaller aerosols are more likely to penetrate the deep alveolar spaces (and hence gain closer proximity to the blood) than are viruses transmitted in larger droplets (Halpin, 1975).

One thinks of the skin of animals as a very effective barrier to viruses except when wounds occur; but this is not an infrequent occurrence in domestic and wild animals. By this means viruses can be transmitted directly into the blood and lymph via the skin. This is probably a common route of infection for papilloma viruses.

Other 'physical' barriers exist even for viruses which have entered the animal by means of a suitable orifice. Thus the trachea is lined with ciliated epithelial cells, and the beating cilia constitute an important first line of defence against respiratory viruses. A more widespread defensive barrier is the basement membrane, which is found surrounding many tissues, and which appears to be a partially effective barrier to viruses entering these tissues from the blood and lymph. Cytomegaloviruses (CMVs) utilize salivary glands as primary targets in their natural hosts, hence their old name: salivary gland viruses. Yet these viruses, as exemplified by the murine CMV, require several days between the time they are disseminated in the blood and the time at which they eventually penetrate the glandular cells. By this time the replication of virus in visceral organs such as spleen and liver is over (Hudson, 1979; Osborn, 1982). Once the virus has established an infection in these glands however, the same basement membrane protects the infected cells from immunological attack (Henson and Neapolitan, 1970).

Phagocytic cells, such as macrophages and neutrophils, can also act as effective barriers to virus infection because of their ability to phagocytose and degrade some viruses. But other viruses, notably herpes viruses, have evolved ways of escaping this barrier, and in some instances actually persist within these cells, e.g. murine CMV (Brautigam *et al.*, 1979; Hudson, 1984).

Many tissues comprise a mixture of virus-susceptible and -resistant cells. Theoretically any step in the virus replication cycle, as outlined in Fig. 4 could be defective in a particular cell type because of the limitation or unavailability of essential host cofactors or processes. Some of these details are only now becoming recognized in cell culture studies. If the resistant cells are stationed at a point of entry to a tissue, then the virus may be effectively blocked before gaining access to the susceptible cells.

One has to remember, in all of these considerations, to distinguish between natural and experimental virus infections. Much information about animal infections has been derived from experimental infections, in which virus has been deliberately inoculated by unnatural routes such as intraperitoneal or intracerebral, and in which the virus might gain access to tissues that do not normally encounter the virus. Although such experimental information is valuable, it could lead to false conclusions when extrapolated to the natural situation.

4.1.2. *Host Genetics*

As indicated before, resistance to virus infection may be manifest at many levels of the host–virus interactions, and many of these manifestations are probably genetic determinants. The most striking demonstrations of resistance are those seen at the level of the whole animal. A classic example is the susceptibility of most farm animals to FMDV, in contrast to the resistance of horses to this virus. The nature of this resistance has not been explained, although such information might be of use in future control programs. Likewise, horses appear to be resistant to pseudorabies virus while all other domestic animals are readily affected. On the other hand vesicular stomatitis virus (VSV) readily produces disease in horses, as well as in cattle and pigs, but sheep are resistant. Thus there is no common denominator which can help to predict in advance whether a given species should be sensitive or resistant to a virus.

Resistance can also extend to individuals within a genus or even a species. For example different species of pacific salmon (genus *Oncorynchus*) differ markedly in their sus-

ceptibility to salmonid viruses (Pilcher and Fryer, 1980a), and some strains of sheep are relatively resistant to bluetongue virus (Sellers, 1981). These considerations have given impetus to the concept of breeding virus-resistant strains of fish and mammals. Unfortunately, resistance to a given virus does not guarantee resistance to other, even related, viruses. A good example of this lack of correlation is afforded by mice infected with herpes simplex virus (HSV) or murine CMV. Both viruses are herpes viruses, yet the spectrum of resistant and susceptible mouse strains is quite different for the two viruses (Lopez, 1975, 1980; Chalmer *et al.*, 1977; Kirchner *et al.*, 1980; Chalmer, 1980). Furthermore, since viruses themselves are continuously changing, a supposedly virus-resistant breed may not remain resistant to newly emerging strains of the same virus. In this context one should be reminded of the 'ecological flux' existing in the classic laboratory model of a bacteriophage T4-infected culture of *Escherichia coli*, in which phage-resistant mutants of the *E. coli*, as they arise, gain a selective advantage in the culture, only to be attacked by mutant phage which have acquired an altered host range (Mathews, 1971). Viruses, like all other forms of life, are continuously changing.

Resistance may also be mediated at other levels of interaction, such as specific cell types, and examples of these are included in the following discussions of genetic determinants.

4.1.2.1. *Histocompatibility genes.* Many attempts have been made to correlate disease susceptibility with specific host genotypes and in particular with the histocompatibility status of mice (H-2 genes) and humans (HLA genes). Extensive studies have been made with several virus infections in mice, e.g. murine hepatitis virus (a coronavirus, MHV) (Bang and Cody, 1980); murine CMV (Chalmer, 1980); and Friend Leukemia virus (FLV) (Specter *et al.*, 1980); and Marek's disease virus (MDV) in chickens (Payne, 1982).

In studies with MHV and MCMV, it has been shown that each virus displays a unique spectrum of relative susceptibility with regard to specific strains of mouse. Thus some strains are fully susceptible, some are resistant, while others are intermediate. The spectrum is different for the two viruses however. H-2 genes are involved to some extent but, at least for MCMV and FLV, non-H-2 genes also contribute to the degree of resistance. In addition the virus genotype can influence the response (Bang and Cody, 1980; Hudson, 1984). Therefore it is not at all clear at present what is the precise nature of the host and virus genes which determine the outcome of an infection. This is partly due to the unknown functions of the H-2 genes implicated, and partly due to the fact that various other relevant host genes may be unwittingly selected for in addition to the obvious marker genes during animal strain derivations. On the other hand specific viral genes which act as determinants should be easier to identify and analyze. In this connection it has become evident that some specific viral genes can act as determinants by virtue of the interactions of their protein products with cell surface receptors, with the result that only the appropriate interaction leads to successful penetration of the cell. Reoviruses represent good examples of this phenomenon, and this aspect will be discussed in more detail in Section 4.2.1.

In addition to the effect of H-2 and other genes on gross resistance or susceptibility to a virus, which is really a reflection of a summation of pathological events leading to mortality or survival, host genes can also determine the type and severity of local pathology. For example, an equivalent amount of MCMV inoculated into different strains of mice gives rise to markedly different degrees of spleen and liver necrosis. The type or degree of response does not correlate with mortality. In fact each mouse strain responds to the virus with a distinctive pattern of disease; in some strains certain tissues exhibit no pathological signs at all (Grundy *et al.*, 1981; Grundy and Melief, 1982).

Some of these manifestations are linked with H-2 gene status while others are not. On this basis it is easy to understand why individual animals within a herd do not all react to a virus in the same way, and why the disease symptoms vary.

Evidence has been amassed from the MHV studies to implicate the macrophage as a specific cell type representing the level at which susceptibility or resistance operates. The situation is not so simple however, since macrophages infected *in vivo* do not respond

identically to macrophages infected *in vitro*; other factors, demonstrable in cultures, can affect the degree of susceptibility.

In the case of MCMV, the situation is further complicated by the fact that virulent and cell-culture attenuated strains of virus are treated differently by macrophages. The latter type is restricted while the virulent strain replicates in the macrophages. Attempts have also been made to correlate H-2 gene determined resistance (to MCMV) with the ability of other cell types to support MCMV replication, notably embryonic fibroblasts and tracheal epithelial cells (Nedrud *et al.*, 1982). The former did not show correlation, although the epithelial cells did. These observations may be related to H-2 gene determined viral receptors on the cell surface. It has been shown that certain viruses, e.g. Semliki forest virus (a togavirus) do require cellular receptors, the presence of which is governed by H-2 genes (Helenius *et al.*, 1978). Consequently in a mouse containing the appropriate H-2 haplotype SFV would not be able to penetrate fibroblast cells, although conceivably other cell types may not be governed in the same way and hence could still be permissive for virus replication.

The importance of the chicken herpes virus, Marek's disease virus (MDV), has led to attempts to breed a virus-resistant strain of chicken. MDV produces a lymphoproliferative disease which rapidly disseminates and yields tumors in neural and other tissues. The virus is also transmitted readily once it establishes an infection within a flock. It appears that relative resistance to the virus is conferred by the B^{21} haplotype; thus breeding chickens with B^{21} should provide a good starting point for MDV resistance, although it is known that other factors, such as sex and age, also influence the degree of resistance (Gavora and Spencer, 1980; Payne, 1982). Nevertheless, a combination of judicious breeding and the application of good vaccines has raised optimism for the prospect of completely containing MDV among commercial flocks. The optimism may wane however in the face of anticipated mutant MDV strains emerging in the future.

4.1.2.2. *Host immune responses.* Many host genes, including some H-2 genes, are involved in determining immune responses to virus infections. More details about these responses will be given in Section 5. In the present context it is sufficient to point out that the consequence of virus infection is largely influenced by the immune responses. It is known from studies in humans that genetic deficiencies in humoral or cell mediated responses can render the host particularly susceptible to certain virus infections. Individuals with defects in humoral immunity are very susceptible to those viruses which are dependent upon a phase of viremia for their dissemination, e.g. togaviruses (McLean, 1980). Arabian horses also fall into this category. These horses are unable to produce gamma globulins and are therefore much more susceptible to some viruses than other breeds of horse (Poppie, 1980). In contrast individuals with defects in cell mediated immunity have great difficulty in coping with those viruses, such as herpes, which tend to remain cell-associated in the body and are relatively resistant to neutralization by circulating antibodies (Rouse and Babiuk, 1979). Few cases of immune deficiencies among livestock have been documented, although it seems likely that they do exist and may explain some of the individual or breed variation in relative susceptibility to virus-induced disease.

Mice have offered more variety in genetically determined immune deficiences. The T-lymphocyte deficient or nude mouse is well known, and has been used frequently in studies of T-cell involvement in virus infections. More recently the beige mutant mouse has become popular in view of its deficiency of NK (natural killer) cell function. These mice are extra-susceptible to several viruses such as MCMV, although the exact nature of the deficiency, and its relationship to interferon production, are still controversial topics (Bancroft *et al.*, 1981).

In addition to genetically determined immune deficiencies, animals do suffer states of transient or chronic immune deficiency. These may be brought about by malnutrition, physiological changes, environmental stressors, or by other viruses and microbes. Under such conditions of immunosuppression (immune depression), animals are likely to be unusually susceptible to virus infections which are normally quite innocuous. This phenomenon will be discussed in more detail in Section 6.

4.1.3. *Age*

Many experiments have been done with mice and other small animals with regard to the effect of age on susceptibility to viruses. In general newborn animals are especially susceptible to virus induced disease, and mortality is commonly seen with a dose of virus to which the mature animal is completely resistant. Resistance is acquired quite rapidly at a certain age in development. To a large degree this acquisition of resistance reflects the maturation of the immune system, which is invariably immature at birth, although other factors may also be involved, for example phagocyte function, which does not necessarily mature in parallel with lymphocyte development. Consequently macrophages from very young animals cannot restrict a virus (such as murine CMV) as efficiently as can adult animal macrophages.

Many other tissues are also not completely mature at birth; consequently maturing tissues may well contain mixtures of fully differentiated and differentiating cells, which may change their relative susceptibility to a given virus (Laporta and Taichman, 1981; Narayan *et al.*, 1983; Gonczol *et al.*, 1984; Spalholz and Tattersall, 1983) during the first few weeks after birth. Another important factor is body temperature, which in newborn animals may be significantly lower than in adults, and can render the newborns more susceptible to disseminated infection.

The foregoing discussion applies to many species of animal including humans. In ruminants however these factors are to some extent overridden by the protective effect of colostrum, which is rich in many antiviral globulins. Thus for their first few days after birth newborn bovines, ovines etc. are protected so long as they continue suckling. There may then be a period of hypersusceptibility to other viruses until the young animal's own immune system is fully developed.

Different species of animal differ markedly in their rate of growth and probably in their rate of immune system maturation. Thus cattle, horses and humans are considered to grow at proportionately similar rates, but sheep, goats and pigs grow much faster. Therefore it is difficult to extrapolate from one species to another, e.g. from mice to domestic animals.

In some cases, a sublethal infection in an immature animal can lead to tolerance. The best known example of this situation is lymphocytic choriomeningitis virus (LCMV), which can infect many animals. Newborn rodents infected with LCMV develop tolerance, a state in which the viral antigens are not recognized as foreign, and as a consequence the virus establishes a lifelong chronic infection. This will be discussed in more detail in Section 7.

In egg-laying animals such as birds and fish, it is again generally observed that the newly hatched animal is more susceptible to virus infections than the adults (Pilcher and Fryer, 1980a,b), although the yolk sac can provide a source of protective antibodies.

Thus all animals go through a phase in which they are particularly prone to virus infections. Serious considerations are therefore justified for the possible intervention by antiviral therapy during this phase. As a start in this direction consideration has been given to vaccinating chicken eggs in order that the newly hatched chick can emerge already protected against some virus infections.

Aged animals also become more susceptible to virus infections, largely because of the diminution of immune responsiveness; but for economically important animals this phase is not often reached.

4.1.4. *Physiological Factors and Stress*

4.1.4.1. *Influence of hormones and prostaglandins.* Many studies have shown that virus replication in cultured cells can be profoundly affected by the presence of hormones and prostaglandins. These effects are generally attributed to alterations in various intracellular biochemical pathways, especially those involving cyclic nucleotides. The end result is a modulation of viral gene expression such that virus replication is then enhanced or inhibited. As one example, herpes simplex virus (HSV) replication in fibroblast cultures

is enhanced by agents which increase the cellular concentration of cyclic-GMP, while inhibition results from increased cyclic-AMP (Stanwick *et al.*, 1977). HSV and HCMV are also affected in different ways by various prostaglandins and hormones added to culture cells (Harbour *et al.*, 1978; Koment, 1985). Since many hormones and other compounds influence biochemical pathways, and even cellular gene expression, in a variety of target cells in the body, it is likely that the net effect on virus replication will reflect the balance of hormones etc. acting upon particular cell types.

Circadian rhythms are also likely to affect the outcome of virus–cell interactions, although there does not appear to have been any systematic study of this. Since circadian rhythms affect the immune system, as well as hormone concentrations, it is difficult to decide which factors are most important in influencing virus replication. It should also be pointed out in this context that these factors can influence not only the acute phase of virus infections, but also persistent infections (see Section 7).

At the level of the intact animal there are many occasions on which they may be subjected to profound physiological changes (Halpin, 1975). These include such events as onset of puberty; egg laying; lactation; molting; sheep shearing (which may be considered as a particularly rapid molt); transfer to a feedlot for fattening; forcing racehorses into training; spawning in fish; migration of fish from fresh to salt water and the reverse; and hibernation. All of these stresses will likely affect acute and chronic infections, and possibly latent infections (by stimulation or inhibition), although only a few of these have been studied. It may be difficult to analyze the cause–effect relationships in these situations, however, since so many biochemical pathways are affected.

Nutrition is also an important factor, though a complex one. It is well known that well-fed animals are generally more resistant to disease, including virus infections. In contrast animals suffering from chronic infections may become nutritionally deprived, due to their lack of drive to feed adequately, and consequently may become more susceptible to other virus infections.

4.1.4.2. *Stress.* The effects of stress upon animal susceptibility to disease are well recognized though ill-defined. At the level of virus–cell interactions, which is the level relevant to virus replication, the effects are mediated by hormones and other chemical modulators of cellular function. The host responses to stressors (i.e. stress-inducing factors) are complex however, and vary according to the nature and duration of the stress (Stephens, 1980; Wedemeyer and McLeay, 1980; Wedemeyer *et al.*, 1984). Various components of the immune system may also be affected by stressors, and these effects will be relayed to the battle against the virus (Solomon and Amkraut, 1981).

Some of the physiological changes noted in the previous section can be regarded as stress-inducing circumstances. Other more artificial stressors have also been recognized to affect the course of virus infections, usually by decreasing host resistance, and possibly by reactivating persistent infections.

In various species, stressors induce the production of specific 'stress' proteins, although the function of these proteins has not been elucidated (Hightower and White, 1981).

One of the most obvious stressors is overcrowding, which is detrimental to most animals. There has been a trend in recent husbandry practice to deliberately overcrowd animals for economic reasons. Two extreme examples are chickens and hatchery-reared fish. Such animals may be chronically stressed and consequently are more susceptible to virus infections. In spite of the insistence by some people that these animals are not adversely affected by crowding, the experimental evidence indicates otherwise (Wedemeyer *et al.*, 1984).

Not all animal species react in the same way to stressors. Sheep for example are much more easily startled than cattle. Pigs become very aggressive territorially when new contacts are made. Therefore all animals cannot be treated in the same way.

One of the most thoroughly studied stressors is shipping fever (transit fever), which is commonly encountered in cattle during transportation from one site to another and is a

very important disease in economic terms. The underlying cause appears to be infectious bovine rhinotracheitis virus (IBRV), a herpes virus, which normally produces mild respiratory infections in healthy adult cattle. In response to the stress of transportation, and the handling and crowding, the virus produces more severe symptoms and in addition renders the animals susceptible to a secondary bacterial disease. The bacterial infection is usually thought to be a species of *Pasteurella* (Yates, 1982). Since herpes viruses frequently cause immunosuppression (see Section 6), then a severe IBRV infection is likely to make the animal hypersusceptible to any opportunistic microbe.

Other environmental stressors will be considered later in this section.

4.2. Viral Factors Affecting Pathogenesis

Viruses are not genetically stable entities; they are continually evolving. Like other genetic systems, viral genomes undergo mutation; recombinational events with similar or dissimilar genomes; gene rearrangements; deletions, etc. Since animal viruses spend most of their time, and replicate, within animal cells, then most of these genetic changes occur in the host cells. This has led to the emergence of numerous so-called strains. New strains of virus appear frequently 'in the field', more often with some viruses than with others. New strains can also be obtained under experimental infections of animals or cell cultures.

Virus variation has several important consequences. The emergence or re-emergence of new strains (or subtypes) is important to viral diagnosticians and epidemiologists, who must continually expand their battery of laboratory reagents. Extensive changes in the virus genotype can lead to phenotypic changes with resultant alterations in virus antigens. A second important consequence is the effect of virus variation on pathogenesis and the ability of the host to recognize and to counteract virus infections. Thirdly, variation must be taken into account in the design of successful therapeutic measures.

4.2.1. *Virulence and Attenuation*

Viruses which are propagated by serial passage through animals often (though not always) retain their virulence, i.e. their ability to inflict pathology and disease, on the host. In contrast when virulent viruses are passed in eggs or cell cultures they frequently become attentuated, i.e. they lose virulence. This loss in virulence may be reflected in a complete abrogation of disease symptoms in the normal animal host, or it may be partial such that the attenuated virus is now pathogenetic in one or a few selected tissues only.

There are numerous examples of the phenomenon of attenuation in the virological literature. In the present context perhaps the most important ones are those which are used as live virus vaccines (see Section 9 for discussion of vaccines). Infectious bovine rhinotracheitis virus (IBRV) and bluetongue virus (BTV) are good examples of viruses which have been attenuated in the laboratory and provide a source of vaccines. In fact attenuated viruses are available for many of the important viruses of domestic animals.

In some viruses virulence can be regained by passing the attenuated strain back through the normal host. Probably the most extreme example of this property is the murine CMV, in which virulence is apparently fully reversible (Osborn and Walker, 1971; Osborn, 1982). If this virus is maintained solely by transferring salivary gland homogenates from mouse to mouse, the virus retains full potency for all murine tissues including salivary glands. However, if the virus is passaged just once in cell culture (i.e. in fibroblast cultures) it becomes attenuated and is no longer able to establish infections in many tissues, except the salivary glands, which still yield normal amounts of virus. Likewise, virus obtained from the spleen or liver is also attenuated and will infect only the salivary glands. Yet once the attenuated virus has been passed through salivary glands again, it becomes fully virulent. To add to the complexity of the situation, more recent studies have revealed that even salivary gland virus released during chronic persistent infection, i.e. after the acute phase, is partly attenuated; but virus which is later reactivated by means of deliberate immunosuppression of the mice is once again fully virulent (Selgrade *et al.*, 1981; Jordan

and Takagi, 1983). The molecular explanations for these changes in virulence are presently unknown, but are under investigation.

Other viruses may show similar behavior under appropriate conditions. Of considerable significance is the possibility that attenuated viruses administered to animals have the potential of reversion to virulence, especially if they persist within the animal, and especially if the animal is immunosuppressed (Osborn, 1981).

The term virulence has been used over the years to describe a rather vague property of a virus which, in combination with compatible host determinants, is able to dictate the pattern and severity of disease. Only recently has it become clear that viral genes alone can be responsible for virulence. Several intriguing studies in progress are attempting to define the molecular basis of virulence. The MCMV virulence-attenuation phenomenon may be explicable in terms of one or a few viral gene functions, although no evidence is available yet to support this possibility. More direct evidence has accumulated from studies with several RNA viruses, which implicate specific viral genes in virulence, and very recent molecular analyses which demonstrated a role for one or more genes in herpes virus neurovirulence.

Reovirus types 1 and 3 produce distinct patterns of CNS disease in mice, and this is largely determined by their S1 gene products. The protein concerned ($\sigma 1$) is the hemagglutinin of these viruses and is situated in the outer capsid. The use of genetic recombinants between type 1 and 3 viruses has revealed that only the $\sigma 1$ protein of type 1 virus allows penetration of that virus into ependymal cells, with the consequence that these cells are destroyed and hydrocephalus results. Viruses containing the type 3 S1 gene cannot produce this result, although a different pattern of CNS degeneration occurs. Thus the S1 gene product alone determines hydrocephalus production, whereas other gene product(s) determine other features of CNS disease (Weiner *et al.*, 1977; Hrdy *et al.*, 1982).

Rabies virus has always been, and continues to be, a threat to livestock and to those humans who must come in contact with domestic and wild animals. As a result of a bite from an infected animal the virus readily gains access to muscle tissue, or sensory organs in the skin, and thereby penetrates unmyelinated nerve fibers. Axoplasmic flow along the nerve fibers allows the virus to enter dorsal root ganglia, then the spinal cord and up to the brain, where the principal damage is done. Relatively little involvement of the blood or other tissues is seen. Damage to the brain is widespread but subtle in the case of wild strains of virus, although the drastic psychological effects are well known. The virus also gains access from the brain to nerves which innervate certain peripheral tissues such as salivary glands, from which successful transmission can be assured (Murphy, 1977).

Recently it was determined that the virulence of rabies virus was attributable to the glycoprotein component of the virion membrane, and furthermore that a specific mutation in the glycoprotein gene could attenuate the virus (Dietschold *et al.*, 1983; Koprowski, 1984). These 'variants' or 'mutants' could be readily identified by their failure to react with neutralizing monoclonal antibodies to the glycoprotein. The specific mutation was localized to amino-acid number 333 in the protein, which is arginine in the virulent strains, but which had been replaced by isoleucine or glutamine in the attenuated strains. Nucleotide sequence analysis confirmed the specificity of the alteration. Presumably these mutations caused configurational changes in the glycoprotein such that the protein could not react properly with the reference monoclonal antibody. In addition the pathogenetic properties of the virus *in vivo* had changed, although the reason for this is presently unknown. Conceivably the altered glycoprotein may have caused difficulty in penetrating certain cell types.

This kind of analysis is obviously important in connection with rabies virus pathogenesis and the design of better vaccines (see Section 9). Attenuated strains of the virus which, like those described above differ only at this single amino acid, would be undesirable as vaccines because of the likelihood of reversion to virulence by mutation. Thus any other amino acid (not necessarily arginine) replacement at this position which would be compatible with the wild-type configuration of the glycoprotein could lead to reversion to virulence. Likewise a different unrelated amino acid substitution elsewhere in the protein

might also restore its 'virulent' configuration. For these reasons it is advisable to use 'vaccine strains' which have several changes from the virulent strains, and which by laboratory tests have been shown incapable of mutation to virulent strains.

However in view of the intrinsic high rate of mutations in RNA viruses (see Section 4.2.5) it is impossible to guarantee against this happening over a long period of time as a result of accumulated mutations. This issue is discussed further in Section 9.

Influenza virus has been the subject of a number of studies aimed at elucidating the viral genetic basis of virulence or pathogenesis (Sweet and Smith, 1980). A relatively simple example such as those described for reovirus or rabies has not been forthcoming, although current molecular analysis of the virion proteins will undoubtedly provide analogous information soon. Sweet and Smith (1980) have pointed out the apparent complexity of the influenza system. Possibly part of this complexity may be due to the inherent difficulty in performing genetic recombinations between viruses which are continuously mutating while undergoing gene reassortments. The consequence of this is that it is difficult to define single gene determinants and furthermore the virus stocks employed may be heterogeneous unless they are frequently cloned.

Molecular analysis of virulence genes has proceeded in another group of viruses with segmented RNA genomes, namely the Bunyaviruses, in which it has proven possible to obtain recombined (reassorted) genomes with different degrees of virulence (Rozhon *et al.*, 1981).

Herpes simplex virus type 1 produces a variety of ocular diseases in rabbits, and it was shown recently that viral genes determine the exact nature of the disease, i.e. whether or not the stroma of the cornea is involved as well as the epithelium, and the severity of disease. By means of genetic recombinations between different strains of HSV-1, and restriction endonuclease analysis, it became evident that one or more viral genes mapping within the 0.70 to 0.83 region of the genome were the determinants (Centifanto-Fitzgerald *et al.*, 1982). The molecular explanation is not known but this may reflect membrane proteins of the virion which determine penetration into selected cells, or transmission across the basement membrane of the cornea.

The same region of the HSV-1 genome is also largely responsible for the neurovirulence of this virus in mice. A mutant of the virus was found which was at least 10 million times less neurovirulent than the wild type virus. This mutation maps in the 0.71–0.83 region of the DNA (Thompson and Stevens, 1983). The gene function(s) have not been identified, although it must be remembered that this region constitutes about 10–13% of the genome, which probably corresponds to between 10 and 15 genes. Thus different proteins may determine neurovirulence and the pattern of ocular disease. More fine-detailed mapping of these gene mutations should pinpoint the individual genes involved.

Of greater immediate interest to veterinarians is the recent molecular analysis of two commonly employed pseudorabies (PRV) vaccine strains. The Bartha and Norden strains of PRV each contain a deletion of about 3% of the DNA between 0.85 and 0.88 map units (Lomniczi *et al.*, 1984a,b). This amount of DNA probably comprises two or three genes, and evidently is largely responsible for the neurovirulence of PRV in chicks. The two vaccine strains are not neurovirulent. Although the vaccine viruses may have acquired other mutations in their genomes which influence pathogenesis, it is important to be able to define the individual virulence genes. Furthermore the presence of deletions in such genes, as opposed to point mutations, would render this kind of vaccine relatively safe in this connection.

A detailed knowledge of those viral genes, and their products, which determine virulence, would certainly help in the design of safer and more effective vaccines.

4.2.2. *Variation of Viruses in the Field*

Some viruses appear to exhibit relatively little change, as shown by the genetic identity of different isolates from different areas or of isolates taken from the same area over a time period. On the other hand some viruses show considerable changes, representing the

emergence of new strains or the re-emergence of older strains, which may be of sufficient magnitude to cause epizootics. The best studied examples of the latter situation are foot-and-mouth-disease virus (FMDV) and influenza A ('flu).

Retrospective molecular analysis of stored FMDV specimens, and comparison with current isolates, has indicated that the virus changes temporally. The changes can be characterized by electrofocusing of viral polypeptides obtained from infected cells and by ribonuclease-T1 fingerprints of the viral RNAs (King *et al.*, 1981; Rweyamamu and Ouldridge, 1982). Moreover these techniques were successfully used to identify the source of virus responsible for a recent outbreak of the disease off the South Coast of England, a subject of considerable controversy at the time (King *et al.*, 1981; Brooksby, 1981). The culprit appeared to be an older strain which had been considered no longer present in the wild, but was still used for producing vaccines in Europe. The vaccine strain was apparently reintroduced into N.W. France where it produced disease, following which the now virulent virus crossed the English Channel. This type of incident could be an excellent mode of reactivating traditional Anglo–French relationships.

Influenza virus A has a well documented history of variation, and some of the resulting strains have been associated with epidemics and pandemics (in humans), and probably to a lesser extent with epizootics, which have been described from time to time in pigs, horses and domestic fowl (Webster *et al.*, 1982). An interesting example of an epizootic was the recent infection by a novel strain of the virus in a population of harbour seals (Geraci *et al.*, 1982). But of greater economic significance was the recent pandemic of 'flu' in domestic fowl which invaded several states in the USA. Chickens and ducks offered little resistance to this new strain of virus, and as a consequence a total of 15 million birds either died directly from the disease or had to be destroyed in an effort to halt the spread of the virus (Bean *et al.*, 1985). The slaughter policy evidently worked and was undoubtedly more efficient than anticipated emergency therapeutic or vaccination measures would have been. This kind of situation is unpredictable and is quite likely to repeat itself in some animal population in the future.

The influenza A virus comprises eight RNA gene segments, which can undergo recombination with the corresponding genes of other strains of the virus. The virion contains seven proteins, of which the hemagglutinin (HA) is the principal immunogen responsible for the elicitation of neutralizing antibody. Mutations occur frequently in all the genes, but when sufficient amino acid changes have accumulated in one of the antigenic regions of the HA, then the virus can no longer be neutralized by existing antibodies in the human population. Consequently an epidemic results. These events are referred to as 'antigenic drift' (Webster *et al.*, 1982).

Occasionally recombination between different HA genes occurs, with the result that some of the recombinant viruses are completely resistant to neutralizing antibody, and consequently have the capacity to cause widespread disease in all non-immune populations. Such events, called 'antigenic shifts', were responsible for the influenza pandemics of 1918, 1957 and 1968. In the 1957 pandemic the new strain also possessed a different neuraminidase gene. Recombinant strains can be recognized by virtue of the electrophoretic mobilities of their RNA segments and virion polypeptides on gels, in addition to traditional serological tests. Three different HA gene 'subtypes' have been detected among human isolates of influenza A, while more than a dozen have been found in mammalian and avian isolates (Webster *et al.*, 1982; Schild, 1984).

Although the HA protein is the principal antigen of the virus in regard to antibody responses to infection, it is not the sole factor in determining relative virulence among the A strains. Thus for a virus strain to be virulent in the human population, an appropriate combination of other viral genes is also required (Sweet and Smith, 1980).

The presence of so many subtypes of influenza A in the animal kingdom, resulting from 'drift' and 'shift', affords tremendous opportunities for generating novel recombinant strains and possible disease. This allows the virus to persist in nature. The relative ease of recombination and generation of new strains was demonstrated a few years ago in experiments conducted with turkeys and pigs. In these studies it was shown that two strains

of virus could simultaneously infect an individual turkey and give rise to a virulent recombinant strain. Likewise in pigs recombinant strains could also arise and these could be transmitted to additional pigs which then suffered disease (Webster *et al.*, 1973).

This information, together with the known relative ease of transmission of this virus among animals and birds (see Section 2), leads one to suspect that a novel recombinant strain of the virus, to which the human or livestock population is not immune, could emerge at any time. Exactly how, where and why the new strains arise is unknown and unpredictable. This makes the task of influenza control by vaccination formidable (see Section 9).

Influenza B and C viruses can also produce mild epidemics, as a result of antigenic drifts. In contrast, pandemics, due to shift, i.e. recombinant strains, have not been recorded. This may be related to the fact that strains B and C do not propagate in mammals or birds (Webster *et al.*, 1982; Schild, 1984).

On the basis of these observations with influenza virus, it might be anticipated that variation by recombination can occur in other viruses containing segmented genomes. This appears to be the case. For example within the Bunyaviridae family of arthropod-borne viruses, each of which comprises a three segment genome of single stranded RNA, those viruses studied are capable of high frequency recombination between corresponding genome segments. In fact some workers consider the possibility that the Bunyamwera complex, consisting of 12 of these viruses, represents a single gene pool, from which environmental factors have selected and maintained a few individual virus types (Iroegbu and Pringle, 1981). This concept equally well applies to any virus with a segmented genome.

In addition to the recombinational events, drifting within each gene evidently takes place too. Drifting, i.e. accumulated point mutations, has been proposed to account for the divergence among individual genes of the three types of reovirus 1, 2 and 3. However selective pressures seem to have resulted in some highly conserved regions in many of the gene segments. The conserved regions include major antigenic determinants (Gentsch and Fields, 1984).

Rotaviruses, members of the reoviridae which are found in all domestic animals, especially in association with diarrhaea, show considerable heterogeneity among their 11 RNA segments. A variety of bovine rotavirus 'strains' have been isolated from diarrhoeic calves, and in fact genetically distinguishable subtypes (revealed by RNA electrophoresis) were recently detected within single isolates (Sabara *et al.*, 1982). This demonstrated that more than one subtype of virus could coexist within an infected animal, although it is not clear as to how these subtypes arose, i.e. by drift within individual RNA segments or by recombination between corresponding segments. It appears from the published data to date that the rotaviruses have a capacity for variation similar to that for influenza A virus, although the differences in their respective modes of replication (see Section 3) may permit different degrees of recombination or reassortment.

It is interesting to note that bovine rotavirus passaged repeatedly in cell culture does not change genetically (Misra and Babiuk, 1980). This can be interpreted to mean that recombination is more likely to be the cause of variation *in vivo* than is drift, although it is not always wise to extrapolate between *in vitro* and *in vivo* situations.

Evidence also exists, from electrophoretic analysis of RNA segments, of considerable genetic heterogeneity within the orbivirus group of viruses, which includes the economically important bluetongue virus (Section 10.6) and African horsesickness virus. These viruses have genomes consisting of 10 segments of double stranded RNA and have apparently exploited their tremendous recombination potential (Gorman, 1979).

In conclusion, those viruses with segmented RNA genomes have the capacity to vary by two types of mechanism, viz. recombination between corresponding segments of two virus strains; and drifting within individual genes. Both methods can produce antigenically novel virus strains within a population of animals or humans.

One should not extrapolate these conclusions too readily to viruses containing non-segmented genomes, however. While the majority of those viruses which have been well

studied do show serotypes, reflecting genetic variation, the degree of apparent variation differs widely. FMDV has already been referred to above. In contrast the salmonid rhabdovirus, infectious hematopoietic necrosis virus (IHNV), seems to show little if any drift over time within a given geographical area, although isolates from different areas do show differences in virion polypeptides (Leong and Barila, 1982). Possibly environmental factors help to conserve selected strains (see Section 4.2.5). It will be emphasized later that one must draw a distinction between mutation and selective adaptation.

Among the DNA viruses, drifting clearly occurs and in some cases has been shown to be responsible for epidemics. A well documented example is that involving human adenoviruses (Wadell *et al.*, 1980). Since most domestic animals also have their species-specific adenoviruses, similar principles probably also apply.

The 40 or so human adenoviruses fall conveniently into six groups. The assignment of individual viruses was originally based on serological cross-reactions, but has recently been vindicated by the more recent testing methods, viz. gel electrophoresis of virion polypeptides; DNA reassociation kinetics; and restriction endonuclease patterns of the viral DNAs. The latter technique in particular has allowed the possibility of extensive epidemiological studies, since within each group the restriction endonuclease pattern for each virus is similar but unique (Wadell *et al.*, 1980; Wadell and de Jong, 1980). Furthermore it has even proved possible to subdivide type 7 adenovirus into four subtypes, 7a, 7b, 7c and 7d, due to subtle differences in their restriction patterns, and to correlate them with past epidemics. Thus in Northern Europe epidemis between 1958 and 1969 were caused exclusively by type 7c, whereas since 1970 epidemics have been exclusively due to type 7b (Wadell *et al.*, 1981a,b). There does not appear to be any correlation, however, between specific subtypes and disease symptoms. Rather there appear to be fluctuations in the prevalent types and subtypes of adenovirus.

Papilloma viruses have been isolated from warts of many animals, but the virtual absence of cell culture systems for growing these viruses *in vitro* hindered their study until recently (Lancaster and Olson, 1982; Danos *et al.*, 1984). It is feasible however to isolate them in substantial amounts from warts and to analyze their proteins and DNA genomes in detail. Five genetically distinct types of bovine papilloma-viruses have been isolated and characterized (BPV 1-5), and interestingly there is a strong correlation between virus type and lesion type or oncogenetic tissue tropism. These, and papillomaviruses from several other species contain significant regions of nucleotide sequence homology, although the larger fraction of each DNA genome is quite distinct. This had led to the concept of divergence within species of a common ancestral papilloma virus.

It should be noted that, as a result of the successful technique of restriction endonuclease typing, more individual papilloma virus types are being recognized. For example more than 20 distinct human types have recently been documented (Zur Hausen *et al.*, 1984).

The herpes viruses in general have shown remarkably little tendency to vary in nature. Restriction endonuclease analysis of infectious bovine rhinotracheitis virus (IBRV) isolates from numerous cattle infections in Saskatchewan (Misra *et al.*, 1983), revealed the presence of types but no variation within types, and no correlation with disease symptoms. Pseudorabies virus (pig herpes) isolates, and feline herpes virus isolates from different countries showed identical DNA restriction patterns (Herrmann *et al.*, 1984a,b). It is conceivable that for viruses such as herpes, which have a propensity for lifelong latency, genetic drift offers no selective advantage. Nevertheless hints have been thrown elsewhere in this review that cytomegaloviruses may vary during chronic infection. Thus genetic stability may be a feature of only some herpes viruses. Alternatively, the prolonged controlled growth that occurs during CMV chronic infection within the animal may select for variants, whereas HSV, IBRV, and other herpes viruses which exhibit true latency, i.e. no replication, do not undergo variation. This could be a simple consequence of mutation frequency. Thus, the more rounds of replication a viral genome goes through (as for example the prolonged chronic infections characteristic of CMVs) the greater the rate of mutation. But the potential for variation is definitely present, since studies with equine

herpes virus type 1 showed that it does change on repeated passage in animals (Allen *et al.*, 1983a,b).

The presence of multiple types of herpes virus within an animal species might be argued to indicate variation within the host population. However the majority of herpes viruses show no DNA sequence homology, with the exception of the human herpes simplex viruses 1 and 2, which share about 50% of their sequences, and some of the equine herpes viruses and simian herpes viruses which share smaller percentages of sequences within their groups (Hayward *et al.*, 1984). For example the five bovine herpes are genetically distinct (Ludwig, 1984). Possibly this means that these five bovine viruses arose from five independent adaptations to bovine species from different ancestral herpes viruses.

The possibility of recombination between herpes viruses exists, in view of the documented experimental generation of HSV-1 and HSV-2 recombinants, and the ability to perform marker rescue experiments (Halliburton, 1980). The HSV, equine and monkey related types just mentioned may have arisen by recombination. A similar origin may be suggested for the murine CMV, which possesses a marked difference in % GC content between the two halves of the genome (Mosmann and Hudson, 1974). In this case one could propose that recombination occurred between a high GC and a low GC containing virus, and that only the present MCMV has survived to this day.

4.2.3. *Laboratory Variation of Viruses*

As a result of numerous studies involving cultivation of animal viruses in cell cultures and in laboratory-reared eggs and animals, it is clear that, in general, viruses change with time. The phenomenon of attenuation, or loss of virulence, and its value for vaccine production, has already been discussed.

Several mechanisms have been enumerated, viz. (i) variation within the viral genome, i.e. genetic drift; (ii) recombination between viral genomes, a property which can result in the so-called 'antigenic shifts'; (iii) phenotypic variation and the generation of pseudotypes; (iv) production of temperature-sensitive (ts) mutants; and (v) generation of defective interfering (DI) viruses.

The first two mechanisms have already been described above, in connection with field variation. In addition, recombinants between the non-segmented genomes of adenoviruses and SV40 have been derived in cell cultures. These hybrid viruses contain various mixtures of contiguous adenovirus and SV40 genes (Lewis *et al.*, 1969). Depending on the functions of the lost genes, these hybrids may or may not replicate by themselves. There seems to be no reason why such recombinations cannot occur in nature, although, on statistical grounds, the presence of simultaneously replicating viruses within the same cell must be a rare occurrence.

Phenotypic variation assumes a number of forms. One form, referred to as phenotypic mixing, involves infection of a single cell by two different viruses. Occasionally, one virus genome is packaged into a capsid of the other virus, or in a capsid comprised of a mixture of capsomers of both viruses. This property is also exhibited by some membrane containing viruses, especially vesicular stomatitis virus (VSV) and retroviruses (Choppin and Compans, 1970; Zavada, 1982). In these situations the virus nucleocapsid is enclosed by all the membrane of the other virus. The consequence of these exchanges is that the host range of the novel virus or pseudotype is determined by the capsid or membrane. Thus, if the process occurs in nature it may allow virus genomes to infect abnormal hosts. This could account for the very wide host range of viruses like VSV. However, the frequency of occurrence during natural infection must be very low, since the process requires two distinct viruses simultaneously infecting a single cell, in a tissue composed of millions or even billions of cells. The process could be facilitated when an exogenous virus reactivates, and then recombines with, a latent virus infection such as a retrovirus.

Temperature sensitive mutants of viruses, i.e. those mutants which cannot replicate above a specified temperature, are commonly found in the progeny of laboratory passaged viruses, especially in those isolated from persistently infected cell cultures or animals

(Younger and Preble, 1980; Holland *et al.*, 1982). Although there is no obvious selection pressure for such mutants when they arise, their role in maintaining the persistence of viruses is more apparent. They may well be important in natural infections, and this point is discussed further in Section 7.

4.2.4. *Defective Interfering Viruses*

These frequently arise during long-term cultivation of infected cells. They have been encountered in many virus families, and there is some evidence from animal experiments suggesting their importance in natural persistent infections. The underlying mechanisms involved have not been completely elucidated, but are clearly different for different virus families. The phenomenon was first described for influenza A virus. More recent detailed investigations have been particularly fruitful for VSV and for Semliki forest virus (SFV) (Perrault, 1981; Lazzarini *et al.*, 1981).

The DI viruses, or DI particles, invariably arise on successive infections of cells at very high multiplicities of infection, i.e. inputs of several hundred or more virions per cell. In order to qualify for the designation of DI virus, all of the following criteria must be met: a DI particle: (i) contains only part of the virus genome, i.e. contains a deletion; (ii) contains all or most of the virion proteins; (iii) replicates only in the presence of the homologous non-defective virus; (iv) interferes specifically with the replication of the homologous non-defective virus; and (v) mediates this interference intracellularly.

If cloned non-defective virus is passaged repeatedly in culture, DI particles arise spontaneously and this can lead to cyclic fluctuations in the relative numbers of DI and non-DI particles, until an equilibrium is attained (Huang, 1973). This is illustrated by the somewhat idealized graph in Fig. 5. The number of DI particles can be measured by means of an interference assay. This pattern has been used to explain the establishment and/or maintenance of persistent infections by VSV. One group of workers has been able to maintain VSV infected cell cultures for over 10 years, and in such systems there is a continuous production of infectious particles (of the order of one per 100 cells per day) and of DI particles (Holland *et al.*, 1982).

The DI genomes of VSV, and of some other RNA viruses such as SFV, arise through deletions, although it is not clear why and how they do so. The mechanism of the interference phenomenon has received considerable study, especially for VSV. It appears

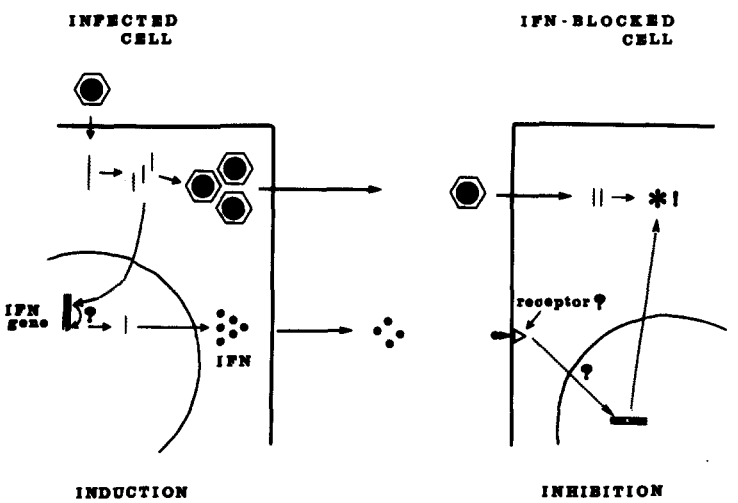

FIG. 5. Induction and action of interferon. The left-hand figure shows a virus-infected cell in which the IFN gene(s) is/are switched on to produce and secrete IFN, concomitantly with virus replication. This cell may die as a consequence of the infection. The right-hand figure shows a neighboring cell protected from virus infection by the IFN, which acts, probably via receptor-mediated transport to the nucleus and derepression of cellular gene(s), to block virus replication. This cell survives, at least temporarily.

that the essential step is the generation of DI(+) RNAs which have the ability to 'sequester' the viral RNA polymerase and hence decrease the overall replication process, resulting in reduced numbers of progeny (−) RNA genomes. The popular explanation for this process is that, during the transit of the replicase along the template (+) strand RNA, the enzyme 'flips' back to the starting point on the template strand, or 'loops' back on the progeny strand, with the result that short pieces of DI RNAs are made continuously, at the expense of full length strands (Perrault, 1981; Lazzarini *et al.*, 1981). The precise molecular details are being worked out, and once the complete nucleotide sequences of the DI-RNAs have been elucidated it should be possible to propose a complete theory for the generation of DIs and their mechanisms of action.

Holland's group has also shown that, following the coinfection of a cell line with complete- and DI-VSV, both of these genomes persisted as anticipated, and in addition both accumulated multiple mutations (especially temperature-sensitive mutations) over several years (Holland *et al.*, 1979).

Rabies virus appears to behave in a similar manner to VSV (Holland *et al.*, 1976; Kawai and Matsumoto, 1977), as does the fish rhabdovirus IHNV in salmonid cell cultures (McAllister and Pilcher, 1974; Engelking and Leong, 1981).

Among the other RNA viruses which have been studied in connection with DI particles are the togaviruses, Sindbis and Semliki forest virus (Eaton, 1981; Barrett *et al.*, 1984; Steacie and Eaton, 1984) and the fish virus infectious pancreatic necrosis virus (MacDonald and Yamamoto, 1977). The mechanism whereby DI particles are generated in these cases are not known but they appear to be different from rhabdoviruses (Perrault, 1981).

DI particles of VSV and SFV have been demonstrated to give protection against acute infections in mice by the corresponding homologous viruses (Doyle and Holland, 1973; Dimmock and Kennedy, 1978). These results have led to the proposal that DI viruses could be used as vaccines, although there are drawbacks to their use, the most obvious of which is the requirement for a DI genome and the complete viral genome simultaneously in the same cell, a situation which would not happen too frequently in a natural infection *in vivo*.

Some DNA viruses can also produce DI particles. For example the polyomaviruses and the adenoviruses, by virtue of their ability to integrate and excise viral sequences into and out of host DNA, can give rise to progeny viruses in which viral sequences have been lost or replaced by host sequences. Consecutive passage of such viruses at high input leads to the generation of DI particles with successively greater amounts of host DNA, the so-called pseudoviruses (Weil, 1978; O'Neill and Carroll, 1983).

Herpes viruses (e.g. pseudorabies virus and equine herpes virus type 1) on the other hand, can generate DI genomes by deleting essential genes and replacing them with repetitive viral sequences (Henry *et al.*, 1979).

From the point of view of the viruses, these schemes are all eminently suited to persistence, since, as long as some non-defective virions remain, then propagation and transmission can also be assured.

4.2.5. *Mutation Frequency in Viral Genomes*

It is worthwhile considering the foregoing discussion in the light of theoretical and empirical arguments concerning mutation rates in RNA and DNA molecules. Significant discrepancy between anticipated and observed rates would imply that additional factors operate in the wild to modify the intrinsic rates.

In general one expects replicating genomes to encounter greater mutation rates than latent or quiescent genomes. The process of replication itself engenders mutations through errors in the copying process. The frequency of errors arising during DNA replication by the polymerase complex has been estimated to be the order of 10^{-6} to 10^{-7} per nucleotide incorporated. But the intrinsic proof-reading mechanism supposedly could reduce this error rate to 10^{-10} (Reanney, 1982; Holland *et al.*, 1982). Viral DNAs which utilize the cellular enzymes, e.g. papovaviruses, adenoviruses, might be expected to suffer similar

rates, and conceivably herpes viruses may do also if they have access to the proof-reading components. Pox viruses on the other hand, since they replicate in the cytoplasm, may not have a proof-reading capacity. Presumably those viruses which exist for a long time as latent infections will suffer relatively few mutations, especially if they reside in non-dividing cells such as neurons.

In contrast RNA genomes can be expected to suffer considerably more frequent mutations for two reasons. The first is the fact that the RNA replicases are relatively 'noisy' (as discussed by Reanney, 1982; and Holland *et al.*, 1982), which means that error rates of 10^{-3} per nucleotide can be expected. This, coupled with the apparent absence of a proof-reading mechanism for RNA replication, implies a vastly greater frequency of mutations for RNA genomes. The second reason is that, with a few possible exceptions, viral RNAs do not exist as true latent infections. Thus generally persistent RNA virus infections are accompanied by some replication, with the result that mutations must continue to accumulate throughout persistence. This became evident from the analysis of Holland's VSV-DI system, in which all of the cloned virus isolates were genetically distinct. A notable exception to this rule is the case of retroviruses, where latency and reduced mutation rates are attained by their existence in the form of proviral DNAs.

Consequently, one should anticipate significant genetic 'drift' during all RNA-virus infections, and, superimposed upon this 'background', additional variation due to recombination or gene reassortment in those viruses with segmented genomes. In fact true recombination (i.e. in the strict molecular genetic sense) has been difficult to demonstrate in RNA viruses, possibly because the rates of mutation are so high. Thus gene reassortment may be the only practical alternative to direct accumulation of mutations.

Estimates of mutation rates for RNA viruses under laboratory conditions has confirmed these arguments. It has long been known that ts mutants, for example, have been much easier to obtain for RNA viruses than for DNA viruses (Reanney, 1982), and reversion rates have always been higher for the former.

More recent oligonucleotide mapping techniques and direct sequence analysis of several RNA viruses such as VSV, influenza, polio virus and others, have also confirmed mutation rates approaching 10^{-3} per nucleotide (Webster *et al.*, 1982). These values were derived from analysis of defined strains of viruses which had been collected over a period of years, or of isolates obtained from persistently infected animals and cell cultures. On the other hand it is also clear that some RNA virus strains appear to have been genetically stable in the wild or in certain laboratory conditions over prolonged periods (e.g. IHNV, Leong *et al.*, 1981; Leong and Barila, 1982). This suggests the presence of extrinsic selective pressures.

Experiments with RNA bacterial viruses have provided a scheme to explain this apparent discrepancy. Evidently these RNA viruses also mutate frequently such that a population of progeny viruses actually comprises a pool of genetically related viruses which compete with each other (Domingo *et al.*, 1978; Eigen, 1983). The most rapidly replicating genome is the one which is naturally selected and this one will prevail so long as the other mutants cannot match its rate of replication. Eventually however it may be displaced by a mutant which has an advantage over the prevailing one (Mills *et al.*, 1967). This scheme of events, as summarized by Eigen (1983), also provides a simple explanation for the success of DI genomes in competing with full length genomes.

One must always remember however, that there is a wide gulf between RNA molecules in a homogeneous aqueous suspension within a test tube and similar RNA molecules sequestered in a complex matrix within a living cell, which itself is continuously bombarded with biological effector molecules. Each conceptual transition, from test tube reaction to RNA phage-infected bacterium, to RNA virus infected eukaryotic cell in culture, to RNA virus infected tissue, represents increasing complexity, increasing divergence from a simple aqueous homogeneous suspension, and an increase in the number of potential intrinsic and extrinsic selection pressures. This argument in no way detracts from the value of the theoretical and empirical arguments outlined above, but we must remember their limitations. If it were possible to compare a given RNA virus in all the four situations just

mentioned, we would probably find that the species of RNA eventually selected would be different in each case, partly because the selection pressures would be different, but also because in the more complex situations the ground rules would be different i.e. there would be some degree of heterogeneity and hydrophobicity in the replication complex. In fact we know that the generation and maintenance of DI RNAs can be influenced profoundly by the host cell (Perrault, 1981; Holland *et al.*, 1982).

A similar situation apparently exists in the VSV persistently-infected cultures; but the system is here influenced by the presence of DI viruses. Thus if VSV is repetitively passaged, *in vitro* or *in vivo*, at high dilution, i.e. low multiplicity of infection, such that no cell will receive more than one virus particle, then a single genotype of the virus prevails if it is suited to the environment. The mutants which do arise simply fail to compete. If however conditions favour the generation of DI genomes, then the equilibrium is displaced in favor of a rapidly replicating DI genome (Holland *et al.*, 1982). Non-defective viruses are also required since the DI viruses cannot replicate autonomously; consequently a standoff situation exists in which both DI genomes and suitable non-defective genomes are preserved. Other selective pressures, such as interferon, can effect the balance, but at any time it is possible to clone out a viable non-defective virus which can initiate acute infection in animals or cell cultures.

Both low multiplicity and high multiplicity infections occur during natural infection. Thus, the initial encounter between the invading viruses and the primary target tissues will most likely result in a low multiplicity infection in which a given cell will receive only a single virion, infectious or defective. This will result in either successful replication or an aborted infection. In contrast the neighboring uninfected cells in the tissue have a high probability of receiving more than one virion per cell, possibly hundreds, from the progeny of the first round of replication. Under these conditions the generation and maintenance of DI genomes will be favored.

These considerations then serve to explain the observations on wild-type viruses. A specific strain of virus can be maintained in nature, not by virtue of lack of mutation, but because it is better suited to the environment than the mutants which arise. If DI genomes are produced at any time however, then a different equilibrium is established.

It has been suggested that this very high mutation rate is a legacy of the primordial nucleic acid copying processes, which may have originally involved RNA only, and which were displaced eventually by the genetically more stable DNA and associated enzymes. One may then ask why such an inefficient scheme would persist until today. Balancing this inefficiency however is the relatively small size of RNA genomes (effectively made even smaller in the segmented genomes), their rapid rate of replication and their high yield of progeny viruses (Reanney, 1982).

The significance of this situation for virus control becomes evident. Unfortunately over the years our understanding of natural virus strains and their variation has been clouded by serological analysis. Virologists commonly refer to virus strains as serotypes, which are usually conveniently 'pigeon holed' for reference. It has become clear however in recent years that a given serotype can often be 'subtyped' on the basis of restriction–endonuclease profiles, in the case of DNA viruses, or T_1-oligonucleotide patterns in the case of RNA viruses. In fact even serotyping leads to increasing levels of subdivision when monoclonal antibodies are employed. Thus, the traditional exercise of classifying viruses into more and more discrete subgroups simply hides the real process of continual evolution, which generates 'pools' of viral genomes rather than discrete 'types'. It has been recognized that viruses which exist as only one or a few serotypes in the wild may still provide considerable heterogeneity in laboratory serotyping analysis and in their ability to produce disease in animals. On the other hand, DNA viruses may be fitted into discrete serotypes more readily only because their rates of mutation are much slower.

In addition rapidly changing viral genomes pose problems for the body's immune system. These can be caused by acquisition of resistance to neutralizing antibody, e.g. a mutation in the antibody binding site, or by acquired resistance to CMI attack, as exemplified by documented cases of mutants which resist the effect of NK cells.

4.3. ENVIRONMENTAL FACTORS

In general domestic animals such as cattle, sheep and pigs are exposed to a greater variation in the environment than humans. The changing weather and temperature fluctuations are therefore of more importance to these relatively unprotected animals. On the other hand broiler raised chickens and hatchery-reared fish are exposed to more uniform environments, although in these situations other stressors become effective, as described above. The environmental factors can be divided conveniently into three categories depending on their effects on: (i) virus survival; (ii) the host animal; and (iii) the vectors.

4.3.1. *Virus Survival*

The most important attribute of a virus is its infectivity for animals. Thus mere preservation of the integrity of a virus is no guarantee of infectivity. The physical appearance of virus (as revealed by electron microscopy) and its chemical composition are not good indicators of infectivity. If as a result of environmental exposure a virus becomes more permeable to water and ions then the virus may rapidly lose infectivity on entering the body fluids.

Temperature is probably the most important single factor influencing viruses, which are expected to be more stable during colder seasons, and in colder aquatic habitats.

Other factors contribute to virus stability, however. Viruses seldom exist as free individual particles, but are invariably associated with organic and/or inorganic material, which itself can affect viruses, both in the atmosphere and in water (England, 1982; Spendlove and Fannin, 1982). The chemical nature of viral capsids and membranes determines their ability to adsorb to such materials. In an adsorbed form they are more likely to retain at least structural integrity. Viruses pathogenic for humans have been detected occasionally downstream from community effluents and in drinking water supplies (Melnick *et al.*, 1978; Payment *et al.*, 1982, 1984). Lakes frequented by birds can be contaminated by influenza A virus (Hinshaw *et al.*, 1978). Also the fish pathogen IPNV has been detected in hatchery effluents (Sonstegard *et al.*, 1972). Water supplies used by and for domestic mammals are therefore a potential source of viruses, the survival of which will depend on the interplay of the factors just described. In this context a concern has been raised about the suitability of sludges and slurries as land fertilizers, on the basis of their possible microbial content (Melnick *et al.*, 1978; Vasl *et al.*, 1983).

The precise physical properties of the air borne droplets responsible for transmitting viruses through the atmosphere affect not only the inherent stability of the virions but also their efficiency of penetration in the respiratory passages of recipient animals (Section 2).

Wind and humidity are additional factors affecting transmission and stability of particle-bound viruses. Forced ventilation in buildings results in renewal of air supplies but on the other hand encourages transmission between animals housed in the same building. In contrast animals in the field tend to remain apart for longer periods (Halpin, 1975).

The importance of climatic factors such as wind and humidity has been well documented in foot-and mouth-disease virus (FMDV) infections. In fact it is now possible to predict the route of spread of FMDV from the source of an outbreak by consideration of the direction and presence of winds and rainstorms (Brooksby, 1981). This is of great help in controlling the spread of disease.

The effect of atmospheric radiation on viruses has received little attention, in spite of the known sensitivity of virus infectivity to u.v.-irradiation. The intensity of radiation reaching the land does vary geographically and seasonally. Short wave u.v. by itself may be insignificant, but the presence of photosensitizing compounds can augment the virucidal effects of radiation (Towers, 1979, 1984; Poulton and Ashwood-Smith, 1983). In addition, many naturally occurring compounds are known which can kill viruses in the presence of long wave u.v. light and visible light (Hudson *et al.*, 1982, 1985a,b,c).

In the aquatic environment viruses are exposed directly to aqueous solutions of various chemicals. The presence of normal concentrations of common salts is likely to preserve

viruses, although many cations even in trace amounts are deleterious. Most viruses prefer neutral pH, and some of them are quite sensitive to pH outside the 6–8 range, whereas others are stable below a pH of 5. In fact, one of the commonest methods for recovering and concentrating pathogenic viruses from water supplies and sewage utilizes exposures to pH values between 3 and 10 (Melnick *et al.*, 1978; Payment *et al.*, 1982). The enteroviruses such as polio are evidently quite stable to these treatments, but the majority of animal viruses are not.

4.3.2. *Effects of Environment on the Host*

Many of the factors discussed above also influence the ability of the host animal to counteract a virus infection, and at least some of these factors can be considered as stressors, which mediate their effects indirectly, through the CNS and immunological mechanisms.

Many species of fish cannot withstand water temperatures of several degrees higher than their normal habitat. Thus rainbow trout kept above 18° become very susceptible to infection by infectious hematopoietic necrosis virus (IHNV) (Hetrick *et al.*, 1979a).

Extremes in weather conditions, and their sudden fluctuations, as occur for example during chinooks or monsoons, can adversely affect domestic animals. Rarer events such as lightning storms, eclipses etc. are also likely to act as stressors. All of these events have the potential for transient immunosuppression and hence increased susceptibility to virus infection, including persistent infections.

The effects of radiation on the animal may be important and, since domestic animals can consume large quantities of plants containing photosensitizing substances, the indirect effects of long wave u.v. and visible light may be more serious than previously thought. The recent recognition of the skin as an important element in the immune system means that any factor affecting the skin has the potential to exert immunomodulation, with subsequent effects on virus infection (Greene *et al.*, 1979; De Fabo and Noonan, 1983). This problem is also relevant to fish inhabiting shallow streams or lakes in summertime. Ulcerative dermal necrosis and sunburn like lesions which occur in these fish have been ascribed to the combined effects of u.v. irradiation and infection by a micro-organism, possibly a virus (Roberts, 1978; Lounatmaa and Janatuinen, 1978; Bullock and Roberts, 1979).

The aquatic environment provides additional stressors for its inhabitants. Most fish are intolerant of low O_2 tension, which is known to be responsible for some cases of virus reactivation in fish which are held in small containers for transportation (Frantsi and Savan, 1971; Roberts and McKnight, 1976; Wedemeyer *et al.*, 1984). Sudden changes in water salinity are stressful. Exposure of trout to sublethal traces of cupric-ion has been shown to render them particularly susceptible to IHNV. Since aquatic habitats everywhere are becoming more contaminated by pollutants, it is likely that fish will be subjected to increasing stress over the next few decades (Wedemeyer *et al.*, 1984).

One cannot over emphasize the effects of overcrowding on the host. Most species of domestic mammals, birds and fish are subjected, at some time at least, to this stressor. In addition, the increased density of the population enhances virus transmission. In fact, one author has aptly likened the typical chicken broiler to a 'living tissue culture' (Halpin, 1975).

4.3.3. *Climatic Effects on Vectors*

In Section 2 the important role of arthropod vectors in the transmission of many animal viruses was emphasized. Some of the environmental factors which have been discussed in this section will also affect the vectors and hence their ability to spread virus. These factors are not necessarily the same for different species of arthropod however. For example mosquitos and ticks inhabit distinctive demographic areas and their life cycles are quite different. Ticks tend to inhabit small areas, sometimes a single animal, whereas mosquitos cover wider areas and spend most of their time in flight.

4.4. VIRUS–HOST INTERACTIONS AT THE TISSUE LEVEL

One aspect of virus–host interactions which deserves greater emphasis is the frequent restriction of viral gene expression within a given population of cells in a tissue *in vivo* and in a cell culture *in vitro*. The former is obviously heterogeneous in terms of cellular morphology and function, while the latter situation is often erroneously thought of as homogeneous. Within a given tissue there are usually populations of cells which are susceptible to a specific virus while other cells are resistant; but even within the potentially susceptible population there are often cells which will not allow replication of the virus, possibly because of constraints imposed by cell cycles, differentiation, or the myriad BRMs (biological response modulators) bombarding tissues. The precise location of a cell in a tissue may also affect its response to the virus. The Reader could probably think of many examples to illustrate this phenomenon, but the Author is most familiar with murine CMV, which seems to prefer distinctive cell types for replication in each of the many tissues it invades (Hudson, 1979). The virus may express some genes in the cells it does not replicate in.

For example in the mouse salivary glands MCMV goes through a complete replication cycle, which is readily evident by histological or electron microscopic examination, in a few acinar epithelial cells lining the ducts, while the vast majority of the cells show no sign of infection (although they might contain viral genomes). In other tissues different cell types are permissive, but again only a fraction of a recognizable population produces virus.

During Epstein–Barr virus (EBV) infection in humans only a very small proportion of the B-lymphocyte population produces virus. This property is carried over into cultured lymphoblastoid cells *in vitro*, in which those few cell lines which do produce the virus restrict its replication to a very small percentage of the population. In this situation the proportion of producer cells may be augmented by various chemicals (Wolf and Seibl, 1984). In some of the non-producing cells, however, variable degrees of viral gene expression may occur (Hayward and Kieff, 1976; Kieff *et al.*, 1982).

It appears that in these circumstances there is a combination of cellular, tissue and viral factors which interact to determine the eventual result in an individual cell, although those factors have not been defined (Sutcliffe *et al.*, 1984).

Any Virologist who has routinely inspected cell cultures for cpe (cytopathic effects) knows that virus production in the individual cells of a culture can be influenced by various intrinsic and extrinsic factors. No such culture is homogeneous; there are position effects, metabolic gradients, variations in O_2 tension, and other factors which together result in different parts of the cell culture being in different physiological stages. Some viruses show little regard for these differences, but many viruses are influenced.

The point of all this discussion is to emphasize the fact that we cannot yet explain in molecular terms the vaguaries of virus–tissue interactions, nor can we predict how even a well studied virus will behave when it encounters a novel tissue. We do not even know to what extent the final outcome is dependant upon host factors and viral factors. Since we do know that viral genes can affect virulence in definable molecular terms (Section 4.2.1), then it is conceivable that virus mutants when they arise and propagate may behave in unpredictable ways when introduced into an animal population. Although it is too early to discuss in specific terms, this does open the way for future considerations of appropriate BRMs at antiviral measures.

5. ROLE OF THE IMMUNE SYSTEM AND INTERFERONS

In broad terms the immune system among vertebrates is similar, especially in regard to immune responses to virus infections. Workers in the veterinary field however, unlike their counterparts in human medicine, are faced with considerable variation in the details of specific responses that occur in different species. The mouse immune system, the one understood in greatest detail, is not necessarily representative of all animals. Cattle, sheep, pigs and horses differ from the mouse and from each other in important details, some

of which are relevant to virus infection. Fish are different again, and there are evidently basic differences between different families of fish (Ellis, 1977, 1982; Manning *et al.*, 1982). The fish immune responses are also subject to greater environmental modulation than mammalian responses.

However, in respect of ontogeny of immune responses, one hypothesis states that by reducing ontogeny to a 'physiological' time scale it is possible to relate events between species because of the so-called differentiation clocks (Solomon, 1981).

The purpose of the succeeding discussion is to highlight the responses to virus infection in domestic species on the ground that full understanding of the interplay between viruses and the immune system is essential in order to design the most efficient and practical antiviral measures.

It is convenient to divide the immune system into several categories. Some years ago it seemed reasonable to teach students of virology that there were essentially two components of the immune responses, namely the humoral (B-lymphocyte) responses and the cell mediated (T-lymphocyte) immune responses. But the demarcation between these two has become blurred and in any case is misleading, since they are not distinct. Any element of the immune system must be viewed as part of a homeostatic system, and therefore a perturbation of any one element is bound to affect the entire system to some degree.

The discussion also includes those components of the defence mechanism, such as phagocytic cells and interferons, which are not strictly immune reactions in that they are not elicited by specific antigens. Nevertheless they are important components of the body's antiviral machinery, and in principle could be manipulated to the benefit of the host.

5.1. Phagocytic Cells

Animals contain several types of cell that are capable of phagocytosing non-specifically micro-organisms, including viruses. It is generally assumed that, because these cells are found in strategic locations in the blood, lymph and tissues, that they act as 'guardians', protecting against dissemination of viruses. In some instances they may function efficiently in this manner. Indeed these cells are efficient in ingesting virions. Unfortunately many viruses spread through the body, not as free particles, but in association with other cells, which can thus gain entry past the phagocytic cells. Furthermore ingestion of viruses does not guarantee their destruction, since many viruses are capable of persisting within macrophages, in potentially infectious forms. Among the viruses which have been studied in some detail in this connection are African Swine Fever virus (ASFV) and murine CMV.

Various types of pig macrophage are susceptible to ASFV infection and the virus can be grown to quite high titers in cultures of such cells. Monocytes are more susceptible than the more differentiated macrophages. Surprisingly, attenuated virus is even more efficiently replicated than virulent virus (Wardley and Wilkinson, 1977). However both virulent and attenuated ASFV establish persistent infections in the surviving cells. Thus macrophage-like cells could be responsible for persistent infection in the pig, or, for that matter, in other carrier animals.

Murine CMV also grows readily in murine macrophages, although the yields of virus are not impressive (Mims and Gound, 1978; Loh and Hudson, 1980; Shanley and Pesanti, 1983). The virulent virus grows to higher titers than attenuated virus, and consequently is able to spread more easily to adjacent non-macrophage cells. Thus macrophages ingest the virus easily but provide little impediment to the progress of the virus through tissues. This virus, like ASFV, is able to persist in some macrophages and can subsequently be reactivated from them (Hudson *et al.*, 1978; Brautigam and Oldstone, 1980).

Therefore in these two instances, and probably many other virus infections as well, the macrophage does not act as an efficient barrier to virus dissemination, but in fact serves as a reservoir for future reactivation.

Neutrophils can ingest and destroy viruses by a well characterized lysosomal enzyme system, but the possibility of persistence has not been explored (Rouse, 1981).

5.2. ANTIBODIES

In humans and in mice primary virus infections elicit the production of IgM antibodies to viral antigens, followed later and on subsequent reinfections by the production of IgG and IgA (Mims, 1982a). The details have been thoroughly investigated in these species and to a lesser extent in a few other laboratory animals. Only in recent years have significant data accumulated for domestic mammals, chickens and a few species of fish. It is clear however that there is considerable variation among families regarding the prevalent types of globulins and their distribution within the body.

In all the mammalian and avian species examined however, the primary response produces the pentavalent IgM (Butler, 1981). In the fishes examined, this role is taken on by a bivalent form of IgG (Ellis, 1982). The subclasses of IgG show considerable variation in concentration and distribution, and there is also variation in the IgA.

Fetuses of ruminants are particularly susceptible to virus infection in view of the lack of transfer of maternal antibodies across the complex placenta. This may explain the relatively high incidence of abortion in these species. Nevertheless fetal lambs have been shown to produce some kind of immunity to bluetongue virus, and fetal lambs and calves can make interferon in response to viruses (Osburn, 1981). But the principal protection derives from maternal colostrum, which contains antibodies to viruses previously encountered by the mother. Colostrum of ruminants is particularly rich in selected classes of IgG, in addition to the presence of IgM, IgA, and complement (Butler, 1981a,b). Lymphocytes and other cell types are also present. The newborn is capable of absorbing these globulins from the colostrum during the first 24 hr after delivery, but not subsequently; therefore it is extremely important that the newborn is allowed to suckle during this period. As the antibodies decay over the next few months the young animal becomes more susceptible to virus infection until it can produce its own. However the time course of maturation of the antibody producing system varies between species.

A genetic defect in globulin production has been detected in Arabian horses, which are unusually susceptible to those viruses which are normally controlled by humoral antibodies (Poppie, 1980).

Antibodies have an additional important role to play in helping to eliminate virus infected cells. They augment the activity of cytotoxic cells in the so-called antibody-dependant cytotoxic cell (ADCC) reaction (Sissons, 1984). This reaction has been shown to be an important early response of cattle to infectious bovine rhinotracheitis virus infection (Rouse and Babiuk, 1979). The mechanism is depicted in Fig. 6. Neutrophils and macrophages, and possibly other cell types, can participate, and only traces of circulating antibodies are needed (Grewal *et al.*, 1977).

The presence of antiviral antibodies, however, is no guarantee of neutralization of a virus. In some cases, circulating antibody does not effectively bind to the virus or, if it does, the virus can remain infectious (Mandel, 1979; Possee *et al.*, 1982; Dimmock, 1984). This situation has been well documented in some instances of lymphocytic choriomeningitis virus (LCMV) infections in mice, in which the virus is chronically tolerated in the face of continuous antibody production (Lehmann-Grube *et al.*, 1983). In this situation, and in other documented chronic infections, virus–antibody complexes may be continuously deposited in the basement membranes of key tissues, e.g. kidney glomeruli, with the result that inflammatory responses are elicited, followed by pathological consequences (Oldstone, 1984a). It is not yet clear what determines successful neutralization of a virus by antibody.

Another intriguing detrimental effect of antibodies (gamma globulins) is the phenomenon recently referred to as antibody-dependant enhancement (ADE) (Porterfield and Cardosa, 1984). In some infections, notably those of dengue virus and other togaviruses, the circulating antibodies promote penetration of macrophages and other cells by the virus, evidently via a certain class of Fc-receptors on the cell surfaces. This property has been used to explain the dengue-shock syndrome exhibited in individuals following re-exposure to the same virus (Halstead *et al.*, 1980). Apparently many other viruses can produce ADE, at least *in vitro* (Porterfield and Cardosa, 1984).

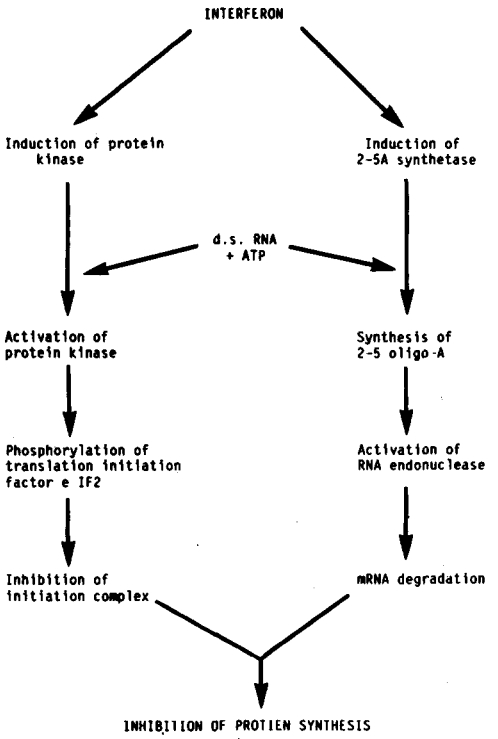

FIG. 6. Proposed pathway of interferon action. Both series of events, which supposedly occur simultaneously, inhibit protein synthesis in the interferon-treated cell.

Teleost fishes possess globulins and react to virus infections by making antibody. There is some controversy as to the types of globulin in the blood, although for the most part they seem to be of the IgM type rather than IgG (Ellis, 1982).

This situation is confused because of the presence of so-called 'natural antibodies', i.e. gamma globulin-like proteins which are found in various species of fish, apparently uninfected, and which react with specific viral antigens. For example, natural antibodies to infectious pancreatic necrosis virus (IPNV) and to channel catfish virus have been detected in fishes (Pilcher and Fryer, 1980a). These could represent authentic antibodies against latent IPNV infections, or some kind of protein which behaves like a globulin during serum protein purification methods, and which happens to have an affinity for IPNV proteins. Natural antibody has been likened to C-reactive protein, a common constituent of fish and mammalian sera which precipitates cell wall materials of micro-organisms (Pilcher and Fryer, 1980a). This would not however explain the antiviral natural antibodies.

In contrast to mammals, where hematopoietic and lymphopoietic tissues reside in bone marrow, the corresponding fish tissues are found in the anterior part of the kidney (Manning *et al.*, 1982).

A critical variable which affects the efficiency of antibody production in fishes is the water temperature (Ellis, 1982). The kinetics of the primary antibody responses to viral and other antigens are invariably slower than in mammals and birds, and even slower in fish inhabiting cooler waters. Rainbow trout for example, require 3–4 weeks at a temperature of 15° in order to produce detectable antibody to foreign antigens. In view of this, one has to question the importance of the primary antiviral antibody response in cold water fishes, especially since the fish viruses themselves replicate within fish cell cultures and in fish tissues *in vivo* with more or less normal kinetics, i.e. similar to analogous mammalian viruses, in spite of the reduced temperatures. The situation is not simple however, since a period of acclimation to a specific temperature before exposure to antigen can accelerate the antibody response (Ellis, 1982).

There are indications that stress, caused by overcrowding, can decrease the antibody responses to IPNV infection. It has been suggested that this immunosuppression is mediated by pheromone-like 'crowding factor' (Perlmutter *et al.*, 1973; Pfuderer *et al.*, 1974).

5.3. Cell Mediated Immunity (CMI)

Cytotoxic T-lymphocytes and natural killer cells (NK cells) have been found in various species of virus-infected animals. In most cases cytotoxic cells have been detected within several days after virus infection, and have therefore been implicated in helping to terminate the acute phase of virus infection, at least in lymphoid tissues (Zinkernagel and Doherty, 1979; Mims, 1982a; Nabholz and MacDonald, 1983; Doherty and Zinkernagel, 1984). More recently NK cells, which unlike cytotoxic T-cells may only be non-specifically augmented by the virus, have been detected in significant numbers within one to two days after virus infection. This has led to the suggestion that the NK cells may be more important in eliminating virus-infected cells early in the infection (Bancroft *et al.*, 1981; Casali and Trinchieri, 1984). In support of this contention is the evidence from studies of the beige mutant mice, which are defective in NK cell activity and consequently are very susceptible to viruses such as murine CMV (Bancroft *et al.*, 1981). The current hypothesis is that NK cell activity is indirectly enhanced by interferon which is induced shortly after infection.

Some studies have questioned the relevance of NK cells in recovery from virus infections, e.g. in Sindbis virus infected mice (Hirsch, 1981). This raises a general issue, i.e. the fact that cells such as NK or cytotoxic T-cells are induced during infection does not necessarily mean that these cells are important in controlling the infection.

Studies on CMI in domestic animals are progressing rapidly. The overall aspects are thought to resemble mouse and human systems, although ruminant CMI is believed to be less important than it is in mice (Schultz, 1981). However, as mentioned in the previous section, NK cells and other cell types can participate in ADCC responses in cattle.

Since the phenomena of delayed hypersensitivity and allograft rejection occur in fish (Ellis, 1982), they have been assumed to possess cell mediated immune mechanisms similar to those in mammals and birds. Fish lymphocytes appear to be divisible into subclasses on the basis of mitogen responses. Hence the general feeling is that CMI in fish probably resembles that found in other vertebrates, although it is probably affected by temperature changes.

5.4. Complement

The constituents of the complement system and the alternative complement pathways are found in mammals, birds and fishes. Complement is able to assist in a number of other antiviral reactions (Rouse and Babiuk, 1979; Sissons, 1984), at least in reconstituted reactions carried out *in vitro*, and there is no reason to suspect any fundamental differences between different animal families.

5.5. Interferons

Interferons (IFNs) are a class of proteins which are synthesized in a wide variety of vertebrate cells in response to virus infections and IFN-inducers. Historically they were regarded as antiviral proteins because of their ability to 'interfere' in the replication of many RNA and DNA viruses (Stringfellow, 1980a; Scott, 1983; Strander, 1983). This property is still used as the basis for their measurement. In more recent years however it has become clear that they have additional functions in connection with regulation of cell growth and differentiation including immunologically important cells (Gresser *et al.*, 1979; Billiau, 1983; Moore, 1983; Taylor-Papadimitriou, 1983). The rate of synthesis of IFNs in normal healthy cells is generally thought to be very low or non-existent. In response to most virus infections or to IFN-inducers, cells synthesize one or more species of IFN which then indirectly protect neighboring cells from virus replication.

A multitude of different IFN types has now been recognized and sequenced as a consequence of recombinant DNA and cloning techniques (Kingsman and Kingsman, 1983; Burke, 1983). In an effort to improve the antiviral efficacy of IFNs hybrid molecules and partly digested molecules are being examined (see Section 9).

According to current nomenclature IFNs obtained from leukocyte cell lines are designated IFN-α, those of fibroblast origin IFN-β, and those derived from lymphocytes as IFN-γ.

The availability of cloned cDNAs for the different IFNs has allowed the determination of IFN-gene mapping and copy number by DNA-hybridization techniques (Burke, 1983). As a result of these studies it has become clear that the human chromosome 9 has more than 12 genes coding for IFN-α and variable forms, plus a single gene for IFN-β, while chromosome 12 has the single gene for IFN-γ. The IFN genes for other animal species have not been so well investigated yet.

Interferon activity is manifest in two discrete phases, the induction of its synthesis and its mechanism of action. Although presumably all cells contain the complete array of IFN genes, their expression is tightly controlled. Embryonic cells (at least in the mouse) do not synthesize IFN in response to inducers, but once extensive differentiation has set in the cells can then be switched on to IFN production. This observation must be relevant to virus infection occurring *in utero*. Similarly, immature or undifferentiated T-lymphocytes do not respond to inducers, whereas their mature counterparts do. In any case IFN-γ is apparently only produced by lymphocytes, not by other cells (Wilkinson and Morris, 1983).

The exact mechanism of induction is not known although it is believed that the α and β genes are to some extent coordinately regulated. Various treatments, known to affect transcriptional events, can influence the level of α and β mRNAs and possibly their translation efficiencies (Burke, 1983).

It should be noted that, in addition to IFN gene products, other genes also respond to inducers, including viruses, although the functions of these other proteins are not known (Lengyel, 1981). Nevertheless, they may also be important in virus infections. This point cannot be over emphasized, since the discovery of IFN itself led to years of erroneous thinking that IFN was only an antiviral molecule, before it was finally conceded by virologists that it had other important functions.

Much less detail is known about IFN-γ, except that its synthesis is induced by mitogens, immune stimulation, and other factors not obviously related to virus infection (Wilkinson and Morris, 1983). Many studies have attempted to define the cell type(s) responsible for IFN-γ production, but conclusions have been difficult to draw because of the heterogeneous nature of most of the cell populations studied. It appears that most classes of lymphocytes can produce IFN-α, and in addition mixed leukocyte populations produce IFN-α and -β. In some cases, at least, cell–cell interactions, e.g. between macrophages and T-cells, may be required.

In regard to the mechanism of action of IFN, there is as yet no unifying hypothesis to reconcile and explain all of the observed antiviral effects. The only general conclusions appear to be that, (i) only one or a few molecules of a particular IFN suffice to 'transform' a cell into an 'antiviral' state; (ii) the effects are mediated via membrane receptors (which may be different for different types of IFN, Fig. 5). After a lapse of several hours, during which time a number of mRNAs and corresponding proteins are synthesized, 'antiviral effects' can be demonstrated (McMahon and Kerr, 1983).

Some viruses, e.g. the papovavirus SV40, are blocked at an early stage in their replication cycle, while many others suffer blocks to transcription and translation. In addition some retroviruses and VSV can be blocked at the stages of virion maturation and release (McMahon and Kerr, 1973). It is clear from the multitude of reported studies that probably no two combinations of cell and virus work in precisely the same way. Most of these studies were performed with cultured cell lines, and it is important to realize that more recent investigations have revealed a tremendous variation between cell types with regard to their basal content of the several key enzymes and substrates considered to be

involved in the IFN-induced mRNA degradation and translation block (see Fig. 6). Apart from this all IFNs have other important effects on cellular structures and functions (Taylor-Papadimitriou, 1983), and many of these may contribute to the fate of the cells, e.g. in determining whether or not the IFN-treated cell will itself survive following blockage of the virus replication.

Figure 6 illustrates schematically the principal components of the so-called '2-5A system' which is commonly activated by IFN treatment of cells (Baglioni, 1979; Lengyel, 1981; McMahon and Kerr, 1983). Among the proteins synthesized, the two best characterized ones are: (i) a protein kinase, which phosphorylates the alpha subunit of the translation initiation factor eIF2, and thereby prevents further initiation of polypeptide chains. This event may not discriminate between cell and viral mRNAs. Conceivably this kinase has other substrates, not yet identified, which affect other cellular processes; (ii) The 2-5A synthetase, which produces the adenosine nucleotide ppp(A2′p)nA and its oligomers ($n > 2$). These nucleotides have the property of interfering with DNA and protein synthesis. Different cells contain markedly different base levels of this enzyme, but the level is invariably elevated by IFN.

Additional components of the 2-5A system include a phosphodiesterase which degrades 2-5A to ATP and AMP; a 2-5A dependant ribonuclease which will degrade RNAs non-specifically; and double-stranded RNA (ds RNA) which augments the entire system (Baglioni, 1979; Lengyel, 1981).

It has been suggested that discrimination against viral RNA can be brought about by virtue of the localized production of ds RNA intermediates during RNA virus replication. Otherwise cellular- as well as viral-directed protein synthesis should be effectively blocked by the combination of sequelae just described in an IFN-treated cell.

To what degree any of this information relates to virus infection *in vivo* is not at all clear. Cells of different types and at different stages of differentiation, which represent precisely what a virus normally encounters upon entering a tissue, might be expected to react in different ways following exposure to IFN. If some of these cells die, then this would provide a convenient way of disposing of the virus. On the other hand, if the blockade of translation is only temporary, then conceivably the virus could subsequently renew its replication cycle in the same cell, or else the viral genome might persist, subject to reactivation much later.

Although the relevance of IFNs to natural virus infections has often been questioned, probably the most important feature supporting their relevance is the speed of the response. Invariably in animal studies IFN induction has been detected very early after virus inoculation, and in some cases has been found in serum within a few hours (Billiau, 1983; Strander, 1983).

Furthermore the administration of anti-IFN antibody to diseased animals has invariably exacerbated the disease, and it has been documented that children who suffer from severe recurrent respiratory virus infection often have defects in IFN production. Therefore IFN would appear to constitute an important early barrier to the spread of virus infection. The possible role of IFN in 'priming' or 'triggering' NK cell activity has also been emphasized by many authors (Casali and Trinchieri, 1984).

However, the induction of IFN by virus can occasionally be detrimental to the host, especially in very young animals. As an example of this problem the lymphocytic choriomeningitis virus (LCMV) induces abnormally high levels of IFN in some strains of newborn mice and, because of the growth regulatory properties of IFNs, these animals suffer decreased growth and development may be runted (Riviere *et al.*, 1980). Problems of this kind must engender care in widespread administration of IFN or IFN-inducers in domestic animals.

Another possible detrimental effect is the known immunosuppressive properties of IFN in various lymphoid cell culture systems (Moore, 1983). Thus, the infected animal may be subjected to two opposing attributes of IFN, viz. control of virus replication and dissemination on the one hand, and immunosuppression followed by enhanced persistence on the other. These two properties have been well documented for MCMV, which induces

IFN production within 24 hpi in mice, but simultaneously induces immunosuppressive factors (Hudson, 1984) (see Section 6).

As far as is known all animals are capable of synthesizing IFN in response to viruses or inducers. The last point has been contentious, although it appears that limitations of IFN induction have been the result of inefficient action of inducers in some species rather than an inherent inefficiency in the IFN synthesis machinery itself. The possibility of a genetic deficiency is always present however, especially since it is known that some animal cell lines maintained *in vitro* are non-producers of IFN (Burke, 1983).

Fish can also produce IFN, although a comparison between fish and mammalian IFN has not been reported. Of particular interest is the fact that rainbow trout kept at 15° produce substantial amounts of IFN with 2–3 days of infection by infectious hematopoietic necrosis virus (De Kinkelin and Dorson, 1973). This is in marked contrast to the relatively poor antibody response in these conditions, and may indicate that fish, especially in cooler water, rely more on immediate antiviral defences such as IFN than on sluggish immune-specific responses.

6. VIRAL-IMMUNOSUPPRESSION

The term 'immunosuppression' refers to the process by which one or more components of the host immune system are suppressed. Many chemicals are immunosuppressive, usually by virtue of direct toxic effects on specific cell types. In addition many viruses also bring about a state of immunosuppression in the host, and this may be caused by indirect effects upon one or more cell types. The viral induced condition may be temporary or prolonged (i.e. chronic). In no case has the detailed mechanism been worked out for any virus, but it is clear that different viruses act in different ways (Woodruff and Woodruff, 1976a; Virelizier, 1975; Hudson, 1979; Rouse and Babiuk, 1979; Semenov, 1981; Denman *et al.*, 1983).

6.1. Significance of Immunosuppression

A very significant aspect of viral immunosuppression is the fact that the infected host is often more susceptible than usual to secondary infection by other viruses or microbes. This consideration can also apply to animals which have been recently vaccinated with a live virus, since in some cases attenuated strains of virus are just as immunosuppressive as their wild-type counterparts.

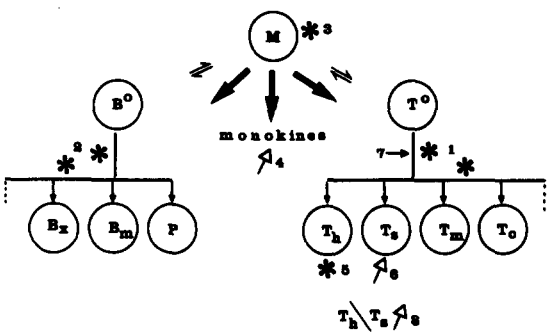

FIG. 7. Viral immunosuppression. The diagram illustrates some of the interactions between monocyte/macrophage cells (M) and B- or T-lymphocytes. Asterisks show points at which certain viruses (numbered) are known or believed to interfere with cell function (cytolytic or decreased function). Open arrowheads show points at which viruses increase a particular parameter. P, plasma cell; Bm, memory B-cell; Bx, other differentiated types of B-cell; Th, helper T-cell; Ts, suppressor T-cell; Tm, memory T-cell; and Tc, cytotoxic T-cell. Examples of categories: (1) T-cell cytolytic, measles virus; vesicular stomatitis virus; minute virus of mice (immunosuppressive strain). (2) B-cell cytolytic; infectious bursal disease virus; vesicular stomatitis virus. (3) Monocyte/macrophage lytic; herpes simplex virus. (4) Increased monokines; murine CMV, Marek's disease virus, African swine fever virus, dengue virus, influenza virus. (5) T-helper cell cytolytic; feline leukemia virus, human T-lymphotropic virus. (6) Increased suppressor cells; avian sarcoma viruses, dengue virus. (7) T-cell growth factor inhibition; avian sarcoma viruses. (8) Decreased T-helper/T-suppressor cell ratio; human CMV, Epstein–Barr virus.

The virus itself may benefit from the condition because it would have the opportunity for greater dissemination within the host than it would in the face of a completely functional immune system. Parenthetically this situation can also be concluded to be beneficial to the host in the long run because of the resulting spread of a vaccine-virus.

In theory, since the immune system is a homeostatic one, any agent which perturbs the steady state may lead to immunosuppression. Thus a multitude of potential target sites are available to a virus. Figure 7 illustrates this diagrammatically. Specific virus examples are inserted into appropriate locations where these are known. Some of these will now be discussed in more detail.

6.2. Examples of Viral Immunosuppression

6.2.1. *Murine Cytomegalovirus*

The murine CMV has been extensively studied in several laboratories, and represents a convenient model for immunosuppression. The MCMV infected mouse suffers a generalized state of immune unresponsiveness, according to a variety of tests for immune function *in vivo* and *in vitro*. These tests are summarized in Table 3. Infected mice have a reduced capacity to respond to antigens and interferon-inducing agents; they accept heterologous skin grafts longer than uninfected mice, and their spleen cells respond poorly or not at all to various T-cell and B-cell mitogens *in vitro* (Loh and Hudson, 1979; Hudson, 1979; Osborn, 1982).

The maximum degree of suppression is reached 3–4 days after infection, i.e. before the peak of virus replication in many tissues and before the onset of tissue pathology. If the dose of virus is sublethal then normal immune functions are gradually restored after about one week of infection, so that by three weeks the animal regains full immunocompetence (Loh and Hudson, 1981).

During this period of suppression the mice are especially susceptible to doses of other micro-organisms which are normally innocuous in competent mice (Hamilton and Overall, 1978). Apparently the MCMV-infected mice cannot clear the secondary invaders. Attenuated virus is equally capable of immunosuppressing mice, although vaccinated animals are resistant to the effects of a second dose of virus. Presumably this means that MCMV must need to multiply in order to immunosuppress (Loh and Hudson, 1980).

There is a correlation between the degree of suppression and the number of infected macrophage-like cells in the spleen. Once the peak of infected cells has been reached (3–4 days p.i.) these infected cells then disappear rapidly and the spleen is repopulated with more normal cells, while immunocompetence is restored. The presence of as few as 100 infected cells in the spleen can give complete abrogration of immune responses (Loh and Hudson, 1982). The virus has little or no direct effect on lymphocytes, however, so the suppression must be due to an indirect effect of the virus.

More recent studies have shown that MCMV infects macrophage-like cells and induces the secretion of an immunosuppressive factor. This factor, which can also be detected in the serum of infected mice, is a small peptide which inhibits the mitogen responses of normal spleen lymphocytes in culture (Hudson *et al.*, unpublished data). It may in fact be a normal immunoregulatory substance, the production of which is considerably enhanced by the virus.

TABLE 3. *Features of MCMV-Immunosuppression*

	Parameter		Response
(1)	Primary or secondary sheep RBC response	*in vivo*	decreased
(2)	No. of antibody forming cells to sheep RBC	*in vivo*	decreased
(3)	IFN induction (NDV; inducers)	*in vivo*	decreased
(4)	Survival of skin grafts	*in vivo*	prolonged
(5)	Delayed-type hypersensitivity reaction	*in vivo*	increased
(6)	Susceptibility to secondary infection by bacteria, fungi	*in vivo*	increased
(7)	Effect of virus dose	*in vivo*	proportional to m.o.i.
(8)	Spleen cell response to mitogens	*in vitro*	decreased
(9)	Spleen cell response to MLR	*in vitro*	decreased

6.2.2. *Marek's Disease Virus (MDV)*

This virus is an oncogenic herpes virus of chickens. MDV infection in chickens produces a general state of immunosuppression which appears to be analogous to MCMV in mice. Thus, infected chickens have a reduced ability to respond to antigens; they temporarily accept allografts; they have impaired delayed hypersensitivity responses; and their spleen cells respond poorly to mitogens. Furthermore the infected animals are particularly susceptible to secondary infections (Lee *et al.*, 1978a,b; Payne, 1982).

Genetically sensitive chickens remain immunosuppressed for several weeks, whereas more resistant animals recover their responses about two weeks p.i. Oncogenic strains of MDV cause considerable destruction of bursal lymphoid tissue, but this is not the principal cause of immunosuppression since the latter can also be caused by non-oncogenic and vaccine strains of MDV, which do not cause lymphoid cell destruction.

Additional studies have shown that infected macrophage-cells are responsible, as in the case of MCMV, although the mechanism is not yet understood. If MDV-leukemic cells are inoculated into chickens, immunosuppression also results, and this has been attributed to the production of suppressor cell populations, including a macrophage-like cell and a T-lymphocyte-like cell (Payne, 1982). However, immunosuppression in tumor bearing animals is probably more complex than in the pretumorigenic state MDV-infected chicken, and suppressor cells may not arise in the latter situation.

6.2.3. *Infectious Bursal Disease Virus (IBDV)*

Another chicken virus, IBDV, is a member of the Birnavirus family. It appears that immature B-lymphocytes, possibly IgM bearing cells, are the primary targets for this virus. Thus, in infected chickens the virus replicates in and destroys a fraction of the bursal cells, and, to a lesser extent, the appropriate B-cells in thymus and spleen (Sivanandan and Maheswaran, 1980a,b; Confer and MacWilliams, 1981). In accordance with this concept the virus replicates in B-cell derived cell lines but not in T-lymphoblastoid cell lines (Hirai and Calnek, 1979).

Consequently infected chickens become defective in gamma globulin synthesis. However T-cell function must also be impaired within several days of infection, since peripheral blood leukocyte responses to the mitogens concanavalin A and phytohemagglutin, and to mixed leukocyte reactions, are depressed. After five days p.i. these responses return to normal. These changes probably reflect the transient lymphoid cell necrosis in thymus and spleen, although the mechanism of this effect is not understood. Macrophages do not appear to play a role in IBDV mediated immunosuppression, hence the mechanism is probably different from MCMV and MDV. Nevertheless infected chickens are unusually susceptible to secondary infection by MDV, Newcastle disease virus, and other micro-organisms. In addition an infection by IBDV around the time of vaccination against MDV impairs the efficacy of the vaccine itself, because the latter cannot elicit sufficient neutralizing antibody (Jen and Cho, 1980).

6.2.4. *Dengue Virus (DV)*

Mice infected with DV type 2 have defective cell-mediated immune responses. Within 2–3 days p.i. there are obvious decreases in spleen weight and numbers of splenic T-lymphocytes. This is followed by a decrease in helper T-cell activity without a corresponding decrease in suppressor T-cell activity. Thus, the ratio of suppressor to helper activities increases, insofar as *in vitro* tests can reflect real function *in vivo*. At the tissue level, T-cell areas of lymphoid tissues show necrosis (Chaturvedi *et al.*, 1980, 1981).

It appears that the T-cells, or at least a resistant subpopulation of T-cells, produce two soluble factors, viz. a prostaglandin like substance which has generalized suppressive effects on immune functions, and a cytotoxic factor (CF). The latter is a protein of about 10^5 MW, which may be bound to a 'carrier' and which is toxic to many T-lymphocytes

and macrophages, though not to B-lymphocytes. Complement is not required for activity. This factor is also secreted by T-cells, and its properties are clearly distinct from the so-called lymphotoxin. The amount of factor increases with increasing time of infection, up to 10–11 days, by which time it can also be detected in the serum. Thus it appears that a fraction of the T-cell population is induced to secrete two factors which together effectively kill or switch off most CMI functions (Chaturvedi *et al.*, 1980).

6.2.5. *Avian Retroviruses*

Many viruses are capable of inhibiting the mitogenic stimulation of murine spleen cells in cultures. One particular group of viruses, the avian retroviruses, is of additional interest because the effect is mediated by ultraviolet inactivated virus and in the absence of penetration into the spleen cells. Thus the inhibitory effect is cell membrane-mediated and does not require viral gene expression (Israel and Wainberg, 1981a,b).

As a consequence of the virus–cell interaction the spleen adherent cells (possible macrophages) synthesize and secrete an inhibitory i.e. 'immunosuppressive' factor. This factor is a relatively unstable protein with a MW of about 20,000, and therefore appears to be distinct from the MCMV and DV-2 induced factors (Israel and Wainberg, 1981a,b).

When this factor is added to spleen cell cultures suppressor cells are produced which in turn can inhibit mitogenesis in additional cultures of untreated spleen cells. The factor is apparently not produced by infected thymus cells.

In addition, more recent work has indicated that the viral immunosuppression may be mediated by interference in the production of T-cell growth factor (Wainberg *et al.*, 1983).

The work cited here represents studies with mouse spleen cells, although it is presumed that, in view of the known immunosuppressive properties of these retroviruses in avian species, that a similar mechanism operates in the natural infection.

6.2.6. *Myxoviruses*

In general those members of the ortho- and paramyxovirus families which have been examined in this respect have been shown to be immunosuppressive. Included are influenza, Newcastle disease virus (NDV) measles virus and rinderpest. More than one mechanism seems to be operative.

Influenza virus interacts with human peripheral blood monocytes and thereby inhibits mitogen responsiveness of lymphocytes (Doyle and Oldstone, 1978). The mechanism may be analogous to MCMV, MDV, DV-2, or avian retroviruses.

In contrast NDV and influenza infection in mice gives rise to a marked alteration in the migratory properties of lymphocytes, i.e. in lymphocyte traffic. The normal traffic flow between different lymphoid organs and blood etc. can be measured by tagging cells with ^{51}Cr and following the movement of labeled cells over a period of time. Conceivably any perturbation in the normal traffic could lead to serious immune deficiencies. In fact this is what happens following brief exposure of the lymphocytes to influenza or NDV. The affected cells migrate preferentially to the liver instead of to spleen and lymphoid tissues, with the result that the latter organs become depleted in some cell types. The effect is reversible however, and the normal traffic patterns are eventually restored (Woodruff and Woodruff, 1976a,b).

The mechanism of this effect is not known but it may involve cell membrane changes resulting from the action of the viral membrane proteins i.e. the neuraminidase or hemagglutinin. Such changes in cell membrane proteins may well influence the cellular recognition signals which direct the traffic patterns.

6.2.7. *African Swine Fever Virus*

This very important virus infection of pigs, an iridovirus, was recently shown to inhibit several B-cell and T-cell immune functions (Wardley, 1982). It appears that the virus

infects monocytes or macrophages and elicits the production of an immunosuppressive factor. Thus the mechanism may be analogous to MCMV and MDV.

6.3. Generalizations About Viral Immunosuppression

It is clear that many viruses cause suppression of immune responses. It is equally clear that one cannot propose a unifying scheme to explain the phenomenon since a variety of different mechanisms seem to operate. These are summarized in Fig. 7. Evidently viruses have exploited various regulatory points in the immune system in order to bring about a more favorable environment for their own multiplication and dissemination. The effects are invariably reversible. In general there are two types of mechanism available: first the direct effect on cells of the immune system, resulting in cell-killing or traffic alteration; secondly the indirect effect in which the target cell, usually a macrophage-like cell, is induced to secrete a factor which in turn affects lymphocyte function. Several different kinds of factor have been detected. They are probably not coded for by the viruses but are more likely to be normal regulatory factors which are induced or amplified as a result of virus–cell interaction.

It is also possible that both general types of mechanism operate simultaneoulsy, as for example during influenza infection.

In addition to immunosuppression, there have been reports of immunostimulation by viral membranes (Goodman-Snitkoff and McSharry, 1982). This process might be similar to the immunostimulatory effects of certain bacteria. The net effect of any viral infection, i.e. the balance between suppression and stimulation, will quite likely be influenced by other factors determined by the host.

6.4. Acquired Immune Deficiency Diseases

Considerable interest and fears have been expressed recently in connection with AIDS (acquired immune deficiency syndrome), a largely fatal disease associated particularly with homosexuals, hemophiliacs, and emigrants from Haiti and Zaire. The underlying cause appears to be a virus, possibly HTLV III (human T-cell lymphotropic virus type 3), which has a profound and selective effect on one or more classes of T-helper cells. The result is a dramatic immune suppression, such that the victim is prone to a variety of secondary microbial infections and other uncontrollable diseases. The virus is apparently transmitted in blood products and certain other body fluids. It seems likely that other host factors are also involved (Conant, 1984; Blattner *et al.*, 1984).

The high risk groups mentioned above would suggest that the disease is exclusively human. Analogous situations have, however, appeared in animal populations. Several colonies of macaque monkeys in USA have been afflicted with an epidemic with similar features of disease. Further investigations revealed the presence of a blood borne infectious agent (Giddens *et al.*, 1979; Henrickson *et al.*, 1983; Letvin and King, 1984). The disease was dubbed simian AIDS (i.e. 'SAIDS').

The feline counterpart i.e. feline AIDS ('FAIDS'), has also been described. In this case the feline leukemia virus, which evidently does kill T-helper-like cells, has been implicated (Butler *et al.*, 1983; Trainin *et al.*, 1983).

Presumably simians and felines have different predisposing social factors from humans. Nevertheless this does mean that veterinarians should be on the lookout for possible AIDS—like epidemics among animal populations. The phenomenon may be more widespread than we have hitherto realized. But clearly blood-borne transmission of the causal agent is not sufficient to explain the disease, unless the agent is extremely rare, since animals in the wild commonly consume flesh and blood of other animals. Further investigations on SAIDS and FAIDS will probably help to unravel some of the tangled problems.

7. PERSISTENCE OF VIRUS IN THE HOST

Until recently it was generally believed that many virus infections, following the subsidence of the acute infection or clinical syndrome, were effectively neutralized by the

immune system and 'eliminated'. In the face of mounting evidence to the contrary however, one has to admit that this scheme rarely if ever operates in nature. The vast majority of virus infections persist in one form or another after the acute stage of the infection. The immune system serves to control the dissemination of the virus, and in some cases at least it probably also helps to force the virus into persistence.

It is important to understand how and where viruses persist in the body, how they are controlled, and to what extent they change their properties during persistence. It must also be recognized that the state of persistence will be determined by: (i) the nature of the virus itself; and (ii) host factors such as the type of tissue and the immune status. Most of these factors have been described in the preceeding discussion already, but they will be recapitulated in the following discussion to bring them into the context of persistence.

7.1. Types of Persistent Infection

Most texts have done little more than concede the existence of persistent virus infections, and in fact many authors seem to have regarded them as oddities. Fortunately Fenner (1968, 1974) has made a serious attempt to classify persistent virus infection, as have several authors of review articles in recent years (Friedman and Ramseur, 1979; Gibbs, 1981b; Mims, 1982b). The most important aspect of these classifications is that they force virologists to think about the concept. These attempts to classify are of course subject to the same pitfalls as any other taxonomic endeavor, i.e. as more viruses and information are added to the system, more and more subclasses are engendered. The main reason for this is that host factors play a prominent role in persistence, such that the exact status of a virus persisting within a given tissue is governed by many parameters, some of which fluctuate.

In spite of this reservation it is worthwhile to use a simple classification, essentially a modified form of Fenner's, and to bear in mind that the apparent boundaries between the categories are blurred and variable (Fig. 8).

The most convenient classification is the use of the three categories: (i) latent infection; (ii) chronic infection; and (iii) slow infection (Fenner *et al.*, 1974). A true latent infection is one in which no infectious virus can be detected but in which some attribute of the virus is recognizable. Thus the definition encompasses all situations in which the viral genome is either dormant without expressing genetic activity or is expressing some or all genes. Historically this state was recognized only by explanting apparently uninfected tissue *in vitro*, where upon a virus sometimes emerged after incubation, or by immunosuppressing apparently uninfected animals, with the same result. In more recent years however, it has

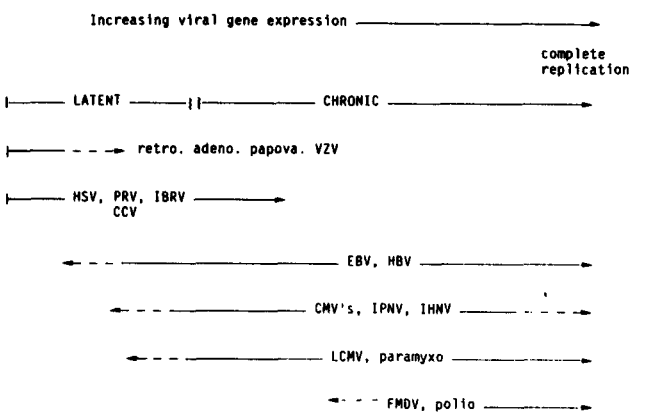

FIG. 8. Spectrum of viral gene expression during persistent infections. VZV, varicella zoster virus; HSV, herpes simplex virus; PRV, pseudorabies virus; IBRV, infectious bovine rhinotracheitis virus; CCV, channel catfish virus; EBV, Epstein–Barr virus; HBV, hepatitis B virus; CMVs, cytomegaloviruses; IPNV, infectious pancreatic necrosis virus; IHNV, infectious hematopoietic necrosis virus; LCMV, lymphocytic choriomeningitis virus; and FMDV, foot-and-mouth-disease virus.

TABLE 4. *Types of Persistent Virus Infection*

(1)	*Latent infections:* No infectious virus demonstrable Viral genome present Can often reactivate *in vivo* and *in vitro* May be viral gene expression Common for DNA viruses, retroviruses
(2)	*Chronic infections:* infectious virus demonstrable in relatively small amounts Usually no tissue pathology Common for RNA viruses
(3)	*Slow infections:* Presence of 'virus' and disease manifest after long incubation period May be due to normal viruses (not intrinsically slow) or virus-like agents

been possible to identify viral DNA, RNA and proteins directly in tissues by sophisticated nucleic acid and immunological techniques. Consequently it has become clear that latent viruses are probably ubiquitous, regardless of the presence of overt disease.

In contrast, a chronic infection is one in which infectious virus is continuously produced, which implies persistent replication. The level of replication may be barely detectable or substantial.

The third type of persistent infection represents those which are categorized as slow because the manifestations of disease require a long incubation period. These may be due to conventional viruses, which are not however intrinsically slow, and to certain virus-like agents (Table 4).

It should be noted that latent and chronic infections may or may not be accompanied by disease, whereas slow infections have only been recognized because of associated disease.

7.2. Latent Virus Infections

The classic example of a latent virus infection, and the one which has received most attention from virologists, is herpes simplex virus. It appears that some animal herpes viruses such as bovine herpes virus 1 (infectious bovine rhinotracheitis virus, IBRV) and pseudorabies virus (PRV) may each exist in a latent form which resembles HSV (Ackermann and Wyler, 1984; Wittmann *et al.*, 1984). Thus, a discussion of HSV latency serves as a useful model for veterinary infections (Fig. 8).

It appears that HSV-1 initially produces an acute infection in the mouth or on the lips, which is self limiting, and is followed by transmission of virions along nerve fibers from the skin to an accessible ganglion. The virus then becomes latent, probably in neurons, and possibly also in glial cells (Wildy *et al.*, 1982). In this state viral genes may be quiescent (Rock and Fraser, 1983). The trigeminal ganglion is evidently a common site for latency, since recent studies on unselected cadavers have shown the present of non-replicating viral DNA in this tissue (Lewis *et al.*, 1984). Similar observations were made recently on feline herpes virus (Gaskell *et al.*, 1985). An infected neuron is an attractive site from the viral aspect, since these cells do not usually divide in adults and they should be relatively protected from immune surveillance. However proponents of the 'dynamic' hypothesis suggest that some virions intermittently travel back down the nerve fibers to the skin where they can renew their replication if the conditions are 'appropriate'. 'Static' hypothesis proponents maintain that the virus remains within the ganglion until the reactivation stimulus. Additional hypotheses, in which virus is alleged to reside continuously or intermittently in the epithelium, have been proposed (Hudson *et al.*, 1976; Hill and Blythe, 1976; Harbour *et al.*, 1983; Klein, 1985).

In either case it is clear that, in response to various stimuli, virus replication at the skin can recur, with the result that a cold sore erupts. It is not known whether the virus initially replicates in the ganglion in response to the stimulus, and then travels to the skin, or if replication follows transmission down the nerve fibre. A substantial fraction of the

population has recurrent cold sores, and some individuals suffer frequent reactivations. A more serious syndrome is keratitis, the ocular form of recurrent HSV disease, in which the cornea is the site of infection (Hollenberg *et al.*, 1976).

The basic situation can be mimicked in animal models such as mice, guinea pigs, and rabbits, and it is clear that other ganglia can serve as sites of latency, depending upon the site of the original infection. Thus, there is nothing unique about the trigeminal ganglion. Its use by HSV is solely a reflection of the fact that many peripheral nerves from the facial skin pass into or through this ganglion (Wildy *et al.*, 1982). Probably other ganglia are also involved, and the virus may well gain access to the brain and establish latency there.

A variety of stimuli have been used experimentally in animals to reactivate the virus, but the only underlying common cause is stress, which involves numerous biochemical changes (Harbour *et al.*, 1983). Some studies have implicated the immune system but in general its role in latency has been questionable (Babiuk and Rouse, 1979; Wildy *et al.*, 1982).

Recent investigations have shown that herpes zoster virus (HZV) establishes similar latent infections in dorsal root ganglia (Hyman *et al.*, 1983), and analogous latent infections of IBRV and PRV exist in bovine and porcine trigeminal ganglia (Wittman *et al.*, 1984). Thus, ganglionic tissue may serve as a common reservoir of latent viruses, especially those of the herpes simplex type.

In spite of many attempts, no suitable cell culture model of latent HSV infection has been obtained, consequently detailed analysis of this type of infection has not been performed.

Many other DNA viruses can establish latent infections in animals or cultured cells, and these commonly persist as integrated viral genes, in which the DNA has become essentially a part of the host genome. The best studied examples of latent infections which rely on integration are the retroviruses. Many of these were originally discovered accidentally as a result of various manipulations of animals or cell cultures. They commonly persist in the proviral DNA form for the lifetime of the host and some can be transmitted vertically through germ-line cells, whereas others can be transmitted horizontally across the placenta or through milk (Weiss, 1982).

Latent infections by RNA viruses have been difficult to obtain, although several examples are known which consist of replicating nucleo-capsids without infectious virus production. Measles virus and other paramyxoviruses have a tendency to persist in this fashion, although some investigators might consider these as examples of chronic infection (Haase *et al.*, 1981a,b; Ter Meulen and Carter, 1982). A recent study has provided evidence of a true latent bluetongue virus infection in cultured hamster cells (Hallum *et al.*, 1984).

7.3. Chronic Virus Infections

Cytomegaloviruses are widespread throughout vertebrates, in which these host-specific viruses commonly persist as lifelong infections. They illustrate well the folly of trying to categorize persistent infections, since it is evident that they can exist as either true latent infections or as chronic infections, depending on the circumstances. In fact they probably exist simultaneoulsy in both states in different cells or tissues (Hudson, 1984).

The mouse CMV has been studied most extensively in connection with pathogenesis and persistent infections. The guinea pig CMV appears to display similar behavior, as do probably all CMVs (Hudson, 1979; Hsiung *et al.*, 1980; Osborn, 1982). Figure 9 summarizes the kinetics of virus replication in several tissues and the accompanying immune responses.

The virus replicates transiently in visceral tissue (as exemplified by the spleen in Fig. 9) and more extensively in submaxillary glands and kidney. A dramatic but temporary immunosuppression is observed after three days. This is illustrated by the abrogation of the concanavalin A response by spleen cells. The immunosuppression appears to be general, and may allow the virus to disseminate throughout the body. However, interferon and natural killer cell activity have been detected shortly after infection (Bancroft *et al.*, 1981; Chong *et al.*, 1983), and eventually circulating antibody and cytotoxic T-cells are

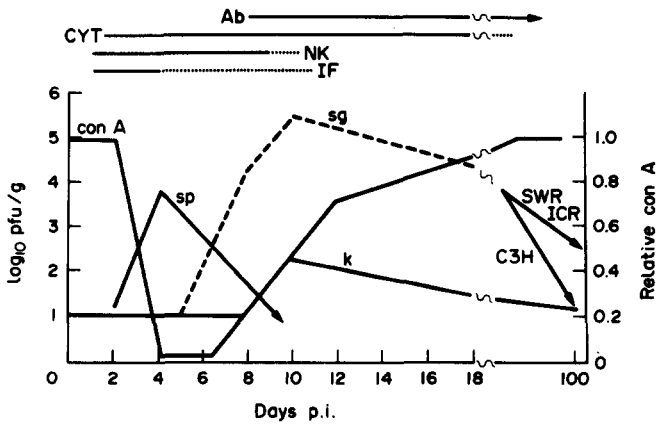

FIG. 9. Murine CMV pathogenesis. Graph summarizes the kinetics of virus replication in key tissues (pfu per gram of tissue; sp, spleen; sg, submaxillary gland; and k, kidney); kinetics of response of spleen cells to the T-cell mitogen concanavalin A (con A); and the specific immune responses to MCMV antigen (Ab, antibody; CYT, cytotoxic T-cells; NK, natural killer cells; and IF, interferon. Dotted lines indicate uncertainty about the precise timing). SWR, ICR and C3H refer to mouse strains. The data have been compiled from various sources, including our own, to give an approximate overall view of pathogenesis (for further details see Hudson, 1979, 1984).

found in abundance. It is not clear how these different responses interrelate, although in concert they do appear to control the amount of virus produced, and may be responsible for terminating the acute phase of infection in tissues such as spleen and liver (Hudson, 1979; Osborn, 1982; Grundy *et al.*, 1981). The virus evidently can enter into a latent state in some of these tissues since it has been possible to reactivate virus from them in some cases, and to detect viral genomes in others long after infection (Chantler *et al.*, 1979; Brautigam *et al.*, 1979; Wise *et al.*, 1979; Brautigam and Oldstone, 1980; Baskar *et al.*, 1983; Hudson, 1984).

7.3.1. *Factors Involved in MCMV Pathogenesis and Persistence*

7.3.1.1. *Strain of virus.* There exist at least two, and probably many more, genetically distinguishable strains of MCMV, viz. the so-called Smith strain, the one originally isolated in 1956, which itself has probably diverged considerably in different laboratories, and the 'virulent' K181 strain selected by Osborn. These two strains differ in their restriction endonuclease patterns and in virulence (Misra and Hudson, 1980), although they do not appear to have been compared in regard to persistent infection.

7.3.1.2. *History of the virus.* Murine CMV obtained from infected mice rapidly attenuates upon passage in cell culture. The resultant attenuated virus retains the capacity to replicate in submaxillary glands (whereupon it reverts to virulence), but not in visceral tissues such as spleen and liver (see Section 4.2.1) (Osborn and Walker, 1971). The attenuation is accompanied by changes in virus protein composition (unpublished data). The submaxillary-passaged virus tends to persist longer in the mouse than does virus repeatedly passaged in fibroblast cultures (Misra, 1977). It is not known if these differences are caused by cultivation of the virus in different cell types. i.e. acinar epithelial cells in the submaxillary gland compared with fibroblasts *in vitro*, or by some other feature of cultivation *in vivo* compared with *in vitro* (Selgrade *et al.*, 1981; Jordan and Takagi, 1983).

7.3.1.3. *Age of mouse.* In common with numerous other viruses, MCMV is much more virulent towards young immature mice than older mature mice. In addition we have found that infected immature mice give rise to a more prolonged chronic phase than do infected adults (unpublished data). This may be explained by a quantitatively greater dissemination

of the virus during the earlier stages of infection, when the immune system is not properly developed, with the result that tissues are seeded with more virus.

7.3.1.4. *Strain of mouse.* A notable host contribution to MCMV pathogenesis is the histocompatibility gene status, which has been studied extensively by Chalmer (Chalmer *et al.*, 1977; Grundy *et al.*, 1981). In general the *b* and *d* haplotypes confer sensitivity to the virus, sensitivity being dominant in crosses. Other non-H-2 genes also contribute however, and Chalmer has argued for the involvement of at least four genetic loci (two H-2 linked, two non-H-2 linked), some of which correlate with specific histopathological effects in certain tissues (Grundy *et al.*, 1981).

The host strain also influences the duration of the chronic infection. This is illustrated in Fig. 9, from which it can be seen that the chronic phase is more prolonged, at least in submaxillary glands, in ICR and SWR mice than in C3H.

7.3.1.5. *Physical barriers.* Conceivably the presence of 'barriers' such as basement membranes could render a tissue or group of cells relatively inaccessible to virus, and to immune responses once infection was estalished. This could explain why salivary glands require a long time to become infected in the first place, and why the infection is then prolonged after this. Henson's group has shown that the duration of the chronic phase in submaxillary glands is inversely related to the efficacy of the inflammatory response. Ultimately the attack on the acini is successful and leads to destruction of infected and adjacent uninfected cells. This process can be alleviated by cortisone, with the result that virus continues to be produced and shed (Henson and Neapolitan, 1970).

7.3.1.6. *Macrophages.* Mims and Gould (1978) have shown that peritoneal macrophages and Küpffer cells can restrict the spread of MCMV, although this restriction was relatively ineffective for salivary gland passaged virus compared with cell-culture passaged virus. This is an important distinction since wild mice presumably are exposed to salivary gland virus. Furthermore, in view of the invariable finding that blood-borne virus is cell associated rather than free virus, then perhaps the importance of the macrophage as a 'guardian' against entry of virus into tissues has been exaggerated. In fact the few macrophages which do become infected *in vivo* may serve as reservoirs for later re-infection. In support of this concept is the evidence that MCMV can persist in, and can be re-activated from, spleen and peritoneal macrophages following infection *in vitro* or *in vivo* (Hudson *et al.*, 1978; Brautigam *et al.*, 1979; Hudson, 1984).

Thus the macrophage may well be an important factor in persistent MCMV infections, but for reasons different from those usually considered relevant to viruses.

7.3.1.7. *Cell differentiation.* Recently Dutko and Oldstone (1981) showed that modulation of gene expression in teraocarcinoma cell lines could affect MCMV transcription and replication, the more 'differentiated' state favoring replication. This is relevant to persistent infections since a cell carrying a latent MCMV genome may lose this control if host gene expression is modulated through differentiation or extracellular factors.

7.3.1.8. *Cell cycle.* Studies in our laboratory (Muller and Hudson, 1977a) indicated that MCMV (like certain other viruses) can only replicate in fibroblasts if they are traversing the cell cycle. Specifically a cellular event associated with early S-phase seems to be required. Other cell types may show a similar requirement. This is relevant to persistent infection since, if a G_0-phase fibroblast carries a latent MCMV genome, then any event which forces that fibroblast into the cell cycle may also permit virus replication.

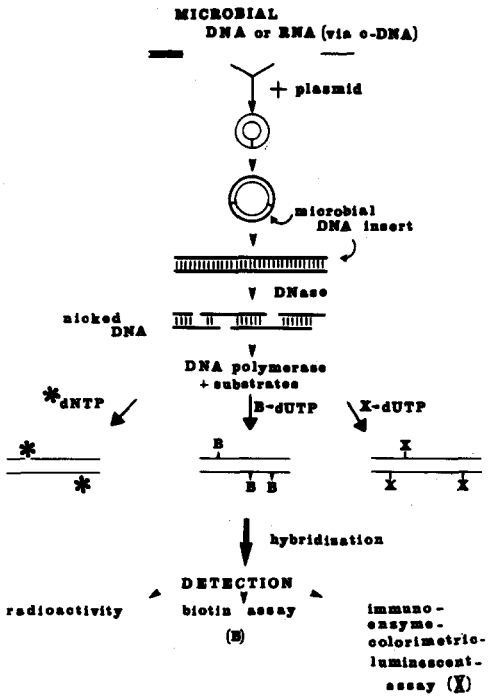

FIG. 10. Preparation of probes for hybridization.

7.3.1.9. *Immune status.* Several workers have shown that MCMV infection enhances NK-cell activity. The importance of this response in the control of infection has been inferred from experiments with beige mutants, which lack significant NK activity and which are especially susceptible to the virus (Bancroft *et al.*, 1981). It is generally assumed that the other immune responses depicted in Fig. 7 are also important in limiting the acute infection, although they do not prevent the establishment of persistent infection. Nevertheless, evidence for a continuing role of the immune system in controlling the virus has been adduced from the many experiments in which immunosuppressive treatments have led to the emergence of infectious virus.

7.3.2. *Reactivation of MCMV*

Many investigators have attempted to reactivate MCMV from a persistent infection, with a view to understanding the mechanisms involved.

Table 5 summarizes the successful attempts.

7.3.2.1. *Reactivation in mice.* It has been customary to assume that, if infectious virus cannot be detected in samples of salivary glands or some other tissues, then the animal must be latently infected (or even uninfected). This assumption may not be valid, for two reasons. Firstly, it is possible that the levels of virus produced are relatively low (1–100 PFU per organ) and hence difficult to detect by standard assay techniques. We have sometimes had to assay entire tissues in order to detect a few infectious particles. Secondly, while the virus may be truly latent in a specific tissue, it may concurrently be shed continuously (i.e. a chronic infection) from other tissues. In fact in our experience it has proven notoriously difficult to obtain persistently infected SWR/J mice which were completely free of infectious virus in all tissues assayed, especially if the mice were immature at the time of infection. Probably in the wild the mothers are relatively young adults and therefore still excrete infected saliva, which then infects the newborns. Under these conditions chronic infections would prevail.

In addition to these problems, individual mice (even of the same strain) do not all exhibit the same duration of the chronic phase, and consequently the result that some mice in an

TABLE 5. *Reactivation of MCMV*

	Experimental material	Reactivation stimulus	References
(1)	*Animals:*		
	Wild mice	antitheta serum	Gardner *et al.*, 1974
	Laboratory infected mice	graft rejection	Wu *et al.*, 1975
	Laboratory infected mice	mock blood transfusion	Cheung and Lang, 1977a
	Laboratory infected mice	cyclophosphamide	Mayo *et al.*, 1977
	Laboratory infected mice	antilymphocyte serum + corticosteroid	Jordan *et al.*, 1977
(2)	*Tissues:*		
	Spleen, lymph nodes Salivary glands, Prostrate	cocultivation with fibroblasts *in vitro*	Henson *et al.*, 1972 Olding *et al.*, 1975 Wise *et al.*, 1979 Cheung and Lang, 1977b Jordan and Mar, 1982
	Embryos	cultivation *in vitro*	Chantler *et al.*, 1979
	Macrophages	'activation'	Brautigam *et al.*, 1979
(3)	*Cell cultures:*		
	Spleen cultures	cocultivation with fibroblasts	Hudson *et al.*, 1979
	3T3 cells	cell cycle stimulation	Muller *et al.*, 1978
	Teratocarcinoma cells	'differentiation'	Dutko and Oldstone, 1981

experiment are entirely free of infectious virus is no guarantee that the others are also free of virus.

These arguments in no way detract from the value of the systems enumerated in Table 5, however, since the reactivation is obviously abrogating some kind of control system, so that virus replication is elevated, either simultaneously in several tissues, or in one key tissue initially followed by dissemination to others. And it still remains a possibility that certain treatments may reactivate the virus from a latent state in a specific tissue in the face of continuous shedding from another. Thus, some of the models in Table 4 may represent models for elevating the chronic infection rather than for reactivating a true latent infection.

With regard to the mechanism involved, the common denominator for reactivation in mice appears to be some aspect of the immune system, since most of these treatments are known to be 'immunosuppressive'. However, one should bear in mind that additional non-immunological 'side-effects' probably accompany all of these treatments, although it is difficult at present to envisage a common non-immunological mechanism. Nevertheless a similar dilemma is posed in trying to deduce the precise immunological mechanism that could be involved, unless this simply reflects the fact that the virus is continually controlled by a combination of immune responses.

7.3.2.2. *Reactivation from tissues.* In these experiments, fragments of tissue or groups of intact cells have been cocultivated with fibroblast indicator cells. Reactivation is indicated by the appearance of characteristic cytopathic effects and the emergence of infectious virus in the indicator cells. Concurrently portions of these tissues may be assayed for free virus. It is probably easier to ascertain the absence of infectious virus in such individual tissues than it is for whole mice, although the problem raised above may still exist.

Earlier experiments were done with spleens and other lymphoid tissues, although more recently similar results have been obtained with submaxillary glands, prostate, embryos, and peritoneal macrophages.

The mechanism of reactivation is not understood. It is unlikely to be a simple loss of controlling factors operating *in vivo*, since the emergence of infectious virus during cultivation *in vitro* usually takes many days. It appears that virus is first released from its carrier cells in an infectious form, rather than as a free genome or nucleoprotein, since its appearance can be inhibited by antibody in the culture medium but not by DNase (Hudson *et al.*, 1978; Wu and Ho, 1979). The virus then spreads in the indicator fibroblasts and possibly any fibroblasts which have grown out from the tissue sample. The initial carrier

cell or latently-infected cell could itself be a fibroblast or some other cell type in which virus replication depends upon the cell cycle. Non-dividing cells from tissues frequently start dividing when cultivated *in vitro*.

This situation appears to be different from the reactivation of HSV from ganglion explants, in which the addition of indicator cells does not help (Lewis *et al.*, 1982).

7.3.2.3. *Reactivation in cell culture.* In order to study the molecular basis of reactivation, we and others have resorted to the use of cell culture models, as indicated in Table 5.

We initially studied spleen cell cultures infected *in vitro*, but it proved difficult to maintain the cultures for prolonged periods of time.

The 3T3 line of murine fibroblasts has also been used. These cells quickly enter a quiescent or G_0-phase as serum growth factors are depleted. The virus readily infects such cells but will only replicate when fresh medium containing serum is added. Under these conditions a substantial fraction of the cell population initiates a cell cycle. The consequent initiation of S-phase is followed by viral DNA replication and ultimately the production of infectious virus (Hudson, 1984).

If the infected 3T3 cultures are maintained in the G_0-phase, infectious virus eventually disappears completely. But the virus can be reactivated at any time, however, by simply providing serum in fresh medium (Hudson, 1984). This system furnishes a useful short-term model for studying the establishment of a latent infection and its reactivation by manipulating the cells' environment.

In further studies we compared the patterns of viral DNA, RNA and protein synthesis in S-phase and G_0-phase cells (Muller *et al.*, 1978; Walker and Hudson, 1986). Unfortunately there are two limitations to the use of this 3T3-cell model. Firstly, cultures maintained in the G_0-phase represent slowly dying cells, for they cannot be propagated, although they may be representative of some cell types in the animal which die after terminal differentiation.

Secondly, different 3T3-cell lines vary somewhat in their response to MCMV. Thus in some of these lines it has proven difficult to block completely the spread of MCMV, which instead establishes a low level chronic infection. This also applies to mouse embryo cultures, although we have been able to demonstrate a correlation of cell cycle traverse with MCMV replication in the latter. This problem may be explained by the difficulty in obtaining a state of 100% G_0-phase cells in such cultures.

A different kind of model used recently was the one afforded by teratocarcinoma cell lines (Dutko and Oldstone, 1981). The virus could be maintained in a more or less latent state in at least one of these 'undifferentiated' cell lines. When such cells were induced to 'differentiate' viral replication ensued. Although it is not clear how the changes taking place in teratocarcinoma cells after induction of 'differentiation' related to infections in defined cells *in vivo*, it is nevertheless interesting and relevant that viral gene expression can be influenced by modulating cellular gene expression.

Another interesting model is that provided by tracheal rings cultivated *in vitro*. These rings can be maintained for long periods of time, and MCMV establishes a long term persistent infection in them. Of particular interest is the finding that epithelial cells serve as the reservoir of chronic virus production, and that the production of infectious virus is dependent upon a continuous low turnover of dividing cells (Nedrud *et al.*, 1982; Nedrud and Wu, 1984).

We have recently investigated a macrophage cell line which shows restricted viral gene expression. Although MCMV replicates in a very small fraction of the cell population ($<0.01\%$), in the majority of cells the viral DNA fails to replicate, yet all early genes are expressed (Walker and Hudson, 1985). Thus the 17 early proteins are synthesized in normal amounts and are processed and distributed in nucleus or cytoplasm normally. The DNA-binding properties of two of these proteins are altered however, although this may not relate directly to the inability of the viral genome to replicate. This system may be relevant to the true latent MCMV infection observed in macrophages *in vivo*.

In conclusion it is clear that many factors, host and virus determined, operate and interplay during the course of MCMV infection. Some of these factors influence the establishment and duration of the persistent phase of infection. The latter represents the net effect of chronic infection in tissues such as submaxillary gland and kidney, and a true latent infection in some other tissues such as spleen.

In the wild the virus seems to have adapted itself to provide for the optimal time of transmission and the greatest degree of persistence.

Reactivation of the virus in mice may represent either an enhancement of a low level chronic infection in some tissues, or a release from a latent infection imposed by constraints operating in other tissues. The immune system has been implicated as a controlling factor, although it is not clear how or at what level it may operate.

The study of several cell culture models has indicated that viral gene expression, and ultimately virus replication, can be governed by the cell cycle in fibroblasts and possibly epithelial cells, and by the cellular gene expression program in other cell types.

7.3.3. *Other Chronic Virus Infections*

An interesting persistent infection by human CMV was obtained by Mocarski and Stinski (1979), who isolated the surviving cells from a human fibroblast culture which had been infected with HCMV. These cells were heterogeneous in that only about 30% of them produced virus. However complete abrogation of virus replication was achieved by culturing the cells in the presence of HCMV antiserum. Under these conditions viral gene expression was restricted, although a substantial number of viral genomes was maintained. When the antiserum was removed, restoration of virus production ensued.

This indicates another mode of persistence, in which the continued presence of antibody may force infected cells into a latent or chronic state. This situation has been investigated in more detail in the case of an RNA virus frequently associated with chronic infection, viz. measles virus. It has been shown that antimeasles antibodies can modulate the expression of certain viral antigens in infected cells and consequently block the production of infectious virions. It has been postulated that similar events may be responsible for forcing viruses into long term persistent infections *in vivo* with disease manifestations, especially in the brain (Ter Meulen and Carter, 1982; Oldstone and Fujinami, 1982).

Many other examples of chronic virus infections of human and veterinary concern could be cited. Many of them represent situations resembling CMV infections, in which virus is continually shed from certain tissues in a fashion controlled or modulated by various host factors, including the immune components. In addition, elsewhere in the animal, the same virus may be partially or completely repressed in terms of viral gene expression, giving rise to latent infections.

An extreme case of variable regulation of gene expression is exemplified by the human herpes virus, Epstein–Barr virus (EBV), which is known to exist in clonally derived B-lymphoblasts in one of several possible states expressing different groups of viral genes (Hayward and Kieff, 1976). At one end of the spectrum the viral genome may only express one or a few genes and resultant antigens, while in other cell clones from different individuals several antigens are expressed and viral DNA may replicate. In the extreme case a few clones even produce infectious virions. The cell populations are heterogeneous however in that a few cells may exist at a different level of control from the others. Furthermore the degree of expression can be influenced by extrinsic factors known to modulate gene expression. The extent of methylation of the viral DNA is one cause of this variation (Wolf and Seibl, 1984).

Thus, any factor which can modulate expression is likely to exert an effect on cells containing DNA and RNA viruses. Since these factors themselves are subject to fluctuation in the body then the exact status of the viral genome may frequently change. Obviously any attempt to classify EBV infection as anything more definite than simply 'persistent infection' would invite controversy.

In addition to this situation there is another kind of chronic infection in which the virus

is 'tolerated' by the host, i.e. it is not recognized as a cluster of foreign antigens. This phenomenon of 'tolerance' has long been recognized in immunology. In the case of viruses, the situation may follow infection of an immunologically immature animal. The best known example is the arenavirus, lymphocytic choriomeningitis virus (LCMV), which infects rodent species and many other animals.

When newborn mice are infected with LCMV, the virus is tolerated and persists lifelong throughout the animal as a chronic infection. Some antibodies are raised and form immune complexes with free virions, but no CMI results; hence the infected cells survive. In the wild the virus is readily maintained by transmission to neonates via various excretions or across the placenta. In this way persistent infection is assured not only within the individual but also within the colony (Lehmann-Grube *et al.*, 1983).

There are complications however, since the immune complexes formed between viral antigens and antibody may be deposited in tissues such as kidney glomeruli, where immunopathology can result. Adults may also suffer CNS disease, to which the official name of the virus refers.

The precise outcome of the LCMV chronic infection is also influenced by host genetic factors, as illustrated by the comparison of different strains of mice. In addition, LCMV produces DI (defective interfering) particles (Welsh *et al.*, 1977), and even some strains of virus which are resistant to the effects of DI particles (Jacobson and Pfau, 1980). In view of the nature of the lifelong persistent replication of the virus, it is hardly surprising that a variety of different kinds of mutants arise, and no doubt these can also influence the state of the infection and disease.

Among the fish and reptilian viruses are several good examples of persistent infections. In fact the majority of these viruses were discovered as a result of reactivating them. Probably the best example is the infectious pancreatic necrosis virus (IPNV), a birnavirus found in many aquatic animal species from shellfish to teleosts. The initial interaction between this virus and a non-immune fish population invariably leads to fatalities, and survivors become lifelong carriers. The infection is clearly chronic since infectious virus is continuously excreted in the feces for years (Wolf *et al.*, 1968; Yamamoto, 1974; Yamamoto and Kilistoff, 1979).

A model experiment accidently performed several years ago provided the best illustration of this. Several lakes in the Rockies of Alberta were unwittingly stocked with IPNV carriers. The resident fish population was infected by the virus, and undoubtedly suffered some losses. The virus was shed from the carriers and was quite likely reactivated in these newly introduced stocks as a consequence of the stress of handling, transportation and exposure to a different aquatic environment. Studies by Yamamoto's group over the next six years showed that the survivors were immune and continued to shed the virus in body fluids, mainly feces, although the incidence of shedding gradually decreased, possibly indicating a waning of the now chronic infection (Yamamoto and Kilistoff, 1979).

Several cell culture models have been described for persistent IPNV and IHNV (infectious hematopoietic virus), and in these the virus appears to be maintained by DI genomes (MacDonald and Kennedy, 1979; Engelking and Leong, 1981; Hedrick *et al.*, 1978).

7.4. Slow Viral Infections

This type of infection is defined as one in which the incubation period is protracted and consequently clinical symptoms require a long period of time, usually years, in order to manifest. These infections are conveniently divided into those caused by conventional viruses, and those caused by unconventional virus-like agents, the exact nature of which is not yet known. They need to be discussed separately because the diseases caused by these two classes of agent are quite different.

7.4.1. *Conventional Viruses*

The viruses which fall into this group are not intrinsically slow, since they characteristically display normal growth cycles in laboratory conditions and some of them produce

common acute infections in their natural hosts. Examples include measles virus, rubella virus and the JC papovavirus, all of which have been implicated in rare progressive CNS disorders in humans (Wolinsky and Johnson, 1980; Ter Meulen *et al.*, 1983; Walker and Padgett, 1983). The prerequisite appears to be the establishment of persistent infection following an inconspicuous acute infection, and possibly host determined factors, so that pathology or immunopathology in specific areas of the brain results.

The veterinarian would be more concerned about the more common infections of sheep and goats by the genetically related members of the lentivirus subfamily of the retroviruses which are responsible for visna, maedi, and progressive pleuropneumonia (Haase *et al.*, 1977). In these infections the viruses persist in various cells of the CNS as proviral DNAs, as expected for retroviruses. In this state the viral DNA can be detected by nucleic acid hybridization techniques, and virus can be rescued by cultivation of brain tissue *in vitro*. In some cells viral gene expression occurs and viral antigens can be detected at the cell surface. Thus, an immune response is mounted. In addition a persistent inflammatory reaction ensues which is probably responsible for the eventual demyelination of the nerves and the consequential clinical symptoms.

In this type of infection it is difficult to envisage antiviral therapy, and vaccination might not prove successful. A more rational approach would be the use of immune-modulators or antiinflammatory agents (see Section 9).

7.4.2. *Unconventional Virus-like Agents*

The classic example of this type of infection is scrapie, which has long been recognized as a transmissible disease of sheep and goats. Vertical transmission also occurs.

It is worthwhile briefly reviewing the recent developments in the study of this agent since this will illustrate the difficulties and frustrations inherent in working with undefined infectious entities (Hunter and Millson, 1977; Kimberlin, 1979; Fraser, 1979).

The incubation period in sheep has been estimated to be about three years, during which time degeneration of neurons commences. This results in progressive brain damage, clinical symptoms and eventually death.

The agent was fortunately adapted to mice, in which the incubation period was reduced from years to months, thus enabling investigators to establish an assay system. Furthermore it became clear that non-CNS tissues could be infected. Numerous attempts to characterize the agent failed. It was clearly infectious, but the infectivity was remarkably resistant to many chemical and physical treatments, including heat and u.v.-irradiation, to which conventional viruses were sensitive. There were controversial and inconsistent claims concerning susceptibility to ribonuclease, deoxyribonuclease and proteases, but no infectious nucleic acid could be isolated. In spite of several attempts it was not possible to fractionate the agent from infected tissues, supposedly because it was strongly bound to membranes. Claims concerning the purification of small virus-like particles have not been substantiated. For a while it was believed that the scrapie agent might be akin to a viroid, which is an infectious DNA or RNA responsible for some common plant diseases (Diener, 1982). But the evidence is against this concept.

In retrospect it is clear that many of the controversial findings reflected the difficulty in working with this unconventional agent. Furthermore some of the experimental interpretations may have been clouded by frustrated virologists who attempted to fit the agent into an existing category.

Similar agents have now been discovered in humans and in mink, in which these rare infections cause progressive degeneration of the CNS. In each case the agent has been successfully transmitted to other animals (Gajdusek, 1984). Their properties are summarized in Table 6. More recently the term 'prion' has been used to designate these agents (Prusiner *et al.*, 1984).

An important feature of scrapie, and similar infections, is the complete absence of immune responses and inflammatory reactions, which implies that the agent is not recognized as 'foreign'. This is important to consider when designing therapeutic measures.

TABLE 6. *Features of Scrapie and Related Infectious Agents*

Agent	Nature	Sensitivity to heat, u.v., enzymes	Transmission	Immune response	Inflammatory response	Pathogenesis
Scrapie	very small virus or nucleoprotein (?)	relatively resistant	vertical or horizontal in sheep—experimentally in many animals	none	none	very long incubation period, degeneration of neurons
Kuru	very small virus or nucleoprotein (?)	relatively resistant	human (cannibalism)—experimentally to various animals	none	none	very long incubation period, degeneration of neurons
Creutzfeld–Jacob (CJ)	very small virus or nucleoprotein (?)	relatively resistant	human (surgery)—experimentally to various animals	none	none	very long incubation period, degeneration of neurons
Transmissible Mink Encepalopathy (TME)	very small virus or nucleoprotein (?)	relatively resistant	mink, consumption of raw meat (conceivably originated from sheep)—experimentally to various animals	none	none	very long incubation period, degeneration of neurons

TABLE 7. *Factors Involved in Persistent Virus Infections*

(1)	Integrated viral genomes. Retroviruses, SV40.
(2)	Episomal viral genomes. EBV.
(3)	Incomplete replication. Measles.
(4)	DI (defective interfering) viruses. Coevolution of DI and Non-DI. VSV.
(5)	ts Mutants. May persist at body temperature. VSV.
(6)	Failure of immunological control, e.g. non-neutralizing Ab, ADE (dengue virus), persistence in macrophages (MCMV), etc.
(7)	Growth in 'protected' sites, e.g. neurons, corneal epithelium, glandular epithelial cells, etc.
(8)	Non-immunogenic agents. Scrapie. Integrated SV40.
(9)	Viral immunosuppression. MCMV.
(10)	Tolerance. LCMV.
(11)	Host defects, e.g. Ab production, NK cells.
(12)	Host and viral genetic factors. H2 or HLA genes. Viral vaccines.
(13)	Cellular restrictions to virus replication, e.g. cell cycle phase; differentiation; cellular factors such as polymerases, regulatory factors.

Another important finding was the recent observation of a common fibrous protein aggregate in tissues of animals infected with all of these prions (Gajdusek, 1984; Prusiner *et al.*, 1984). This proteinaceous material, which apparently resembles amyloid protein, may represent the infectious agent itself or may be a common pathological consequence of the infection. This problem will undoubtedly be solved shortly. At present however it is not clear how the prion replicates. If it is simply a protein molecule then conceivably it may operate by derepressing or switching on one or more cellular genes, giving rise to pathological consequences. Alternatively it may be some kind of aberrant nucleoprotein complex, with pathogenic properties, which has been induced by another infectious agent.

In any case it is clear that viruses (at least the conventional kind) no longer represent the ultimate in simple pathogens. The recognition of subviral infectious agents however promises to provide a new set of problems for those concerned with control measures.

Table 7 summarizes the factors involved in persistent virus infections.

7.5. EFFECTS OF PERSISTENT INFECTION ON HOST CELL FUNCTIONS

In view of the scant attention given to persistent virus infections over the years, it is not surprising that considerations of the detrimental effects of such infections on the host, in the absence of overt disease, have been few. Interest in this subject however has perked recently as a consequence of several intriguing results. It is quite clear that cells which contain chronically replicating but non-cytolytic viruses can suffer profound disturbances in non-essential functions, i.e. those functions which are not essential to cell survival but which constitute an integral part of one or more of the homeostatic systems of the body.

Perhaps the most striking example of this property was illustrated recently by Oldstone's group (Oldstone *et al.*, 1984). They showed that infection of newborn mice with LCMV (lymphocytic choriomeningitis virus) resulted in retarded growth, compared to similar uninfected mice, which was due to a decrease in production of growth hormone (GH) and altered glucose metabolism. This defect was the direct consequence of chronic virus replication, without morphological damage to the cells, in the area of the pituitary gland responsible for GH production. Other pituitary hormones were apparently unaffected.

The defect could be corrected by transplanting cultured GH-producing cells into these infected mice. Evidently the viral-induced malfunction was specific for this one hormone system because of the ability of the virus to exert some kind of specific transcriptional, translation, or post-translational blockage in this cell type.

Needless to say this kind of analysis conjures up hypothetical explanations for many other chronic diseases of unknown etiology. Oldstone (1984b) and Fields (1984) have also reviewed other related observations pertaining to specific viral-induced disturbances in cells with recognizable differentiated functions. Most of these studies involved cell cultures. A notable example is viral interference in acetylcholine secretion in a line of neuroblastoma cells (Rubenstein and Price, 1984). This has obvious relevance to the variety of CNS disorders which are known to involve specific defects in neurotransmitter function.

Undoubtedly this area of study will become popular as virologists and clinical scientists realize the potential impact of covert persistent viruses.

8. LABORATORY DIAGNOSIS OF VIRUS INFECTIONS

Virus infections in domestic animals can occasionally be recognized and presumptively diagnosed by virtue of the clinical syndrome which accompanies the infection. In most cases however, and often including the above situation, it is desirable to perform a laboratory diagnosis. This is especially important if proper control measures are to be adopted. For example a suspected FMDV infection obviously requires prompt confirmation so that emergency measures can be enforced to contain the virus.

The types and methods of specimen collection are beyond the scope of this discussion. These are dictated by standard veterinary practice guidelines, and to some extent by the willingness (or otherwise) of the patient to cooperate.

Once the appropriate specimen has been sent to the laboratory for diagnosis it is then important to perform the tests as rapidly as possible. Unfortunately this is often the limiting factor. In general veterinary virology has tended to lag behind human virology in this regard (although the gap seems to be closing), while fish viral diagnosis has lagged even further. This is partly due to the reluctance of governing authorities to replace some of the conventional and anachronistic techniques with more recent innovative methods, and partly due to the conservatism of the diagnosticians themselves, who are sometimes apprehensive about replacing a time-tested crude method (such as the complement-fixation test) by a novel scientific technique. The cost of a test is also a large factor in determining its acceptability, although methodology based upon the use of monoclonal antibodies and genetic 'probes' will undoubtedly render the newer tests economically attractive in the near future.

8.1. Conventional Diagnostic Techniques

Table 8 represents a summary of the more commonly used tests in relation to their speed, sensitivity and specificity (McLean, 1980; Mohanty and Dutta, 1981). Electron microscopy of negatively stained specimens is by far the quickest technique, and will likely remain so for some years (McLean and Wong, 1984). If substantial numbers of virions are present in the sample, as is often the case for rotaviruses in stools from children or young animals suffering from diarrhea, then the virus can be seen and thus identified within minutes of receiving the specimen. If respiratory specimens contain substantial numbers of paramyxoviruses, e.g. bovine parainfluenza type 3 in nasal secretions, then again identification can be made quickly (in this case independent confirmation might be required).

Unfortunately this technique is not sensitive, since a smaller number of virions can be missed, although it can be argued that this is the only technique which could really implicate the virus as the causal agent of the disease, in view of the sheer number of particles present. Obviously the specificity is also limited since it is usually only possible to identify the virus to the level of its 'family'. Thus all herpes viruses will look the same, and all paramyxoviruses will look the same. Further analysis in these cases would require

Table 8. *Methods of Virus Diagnosis*

	Method	Speed	Sensitivity	Specificity
(i)	electron microscopy—negative staining	++	−	+ (virus family)
(ii)	cell culture isolation—cytopathic effects	−	++	+ (requires confirmation)
(iii)	viral antigens: hemagglutination, ELISA, CF, etc.	± to +	± to +	+ (monoclonal antibodies improve)
(iv)	viral antibodies: two sera required	−	± to +	+ to ++
(v)	DNA probes	± to +(?)	+ (poss ++?)	++ (any level desired)

additional tests. Furthermore electron microscopy can only identify 'virions', without regard for their infectivity.

Cell culture isolation remains the bulwark of viral diagnosis in any field. This technique is generally slow but is exquisitely sensitive, one infectious virus particle being sufficient (at least in theory) to produce a focus of cytopathic effect (cpe) in the appropriate cell culture. The exact nature of the cpe is often a good indication of the virus, especially to an experienced observer. Further confirmation requires an additional serum neutralization or fluorescent antibody test with known antibody. If monoclonal antibodies are used for this purpose, it is important to ensure that they are neutralizing antibodies. Nevertheless there are several major disadvantages to the cell culture isolation technique. One of these stems from its extreme sensitivity. Any cytopathogenic virus that is present in the specimen can be detected, regardless of its role, or otherwise, in the disease. In general however, it is probably reasonable to assume that if such a virus is present in substantial amount then it is a causative agent.

Another disadvantage is the time required for cpe to appear. Some enteroviruses, for example, may show cpe within two days of inoculation into the cell cultures; but others such as CMVs may require two weeks, especially if they are present in small numbers. Still other viruses may require one or two 'blind' passages in cell culture before they finally adapt to the indicator cells and produce cpe. In the extreme situation, some viruses, e.g. the paramyxovirus SV5 (simian virus 5) produce no discernable cpe while they replicate. Of equal concern is the fact that the laboratory must carry numerous cell lines or primary cultures in order to encompass the variety of animal species from which specimens are taken. The human-viral diagnostic laboratory can usually make do with two or three types of primate culture; but obviously the veterinary counterpart must be prepared to deal with a wide range of host specific and broad spectrum viruses. Thus even the best cell culture facility cannot guarantee that the causative virus will be found.

Techniques involving the detection of viral antigens or hemagglutinins are generally faster than cell culture isolation; most of them can be completed within a working day or during the next day (providing the reagents are working properly!). Their sensitivity varies considerably, the ELISA technique being much more sensitive than hemagglutination or complement fixation; but their specificity can be excellent, depending on the reagents employed (Richman *et al.*, 1984a,b; Shamberger, 1984).

These same techniques are also commonly used for the detection of specific antiviral antibodies in sera, by means of the appropriate reference viral antigen. In this situation however the aim is not simply to detect the presence of the antibody in the animal, which by itself means nothing, except that the animal has at some time been exposed to that virus; but rather to detect a significant increase in the antibody titre over a period of one to two weeks. Thus, at least two consecutive serum samples must be tested, ideally representing the 'acute' and 'convalescent' phases of the disease, in order to implicate the virus as the cause. Needless to say, this entire operation spans too much time to be of any use in helping to design therapeutic measures, and the animal may be dead by the time the diagnosis is completed, although this knowledge could be useful in preventing the spread of infection to the rest of the herd. Clearly there is a need for improvement in the analysis of rising antibody titers, so that tests could be made a few days apart instead of the usual one to two weeks.

8.2. NOVEL APPROACHES

For some years molecular virologists have been proposing the use of DNA probes for the detection of viruses in clinical specimens (Pagano, 1975). Until recently however, this approach was spurned on the ground that the tests would be time consuming, too expensive and too hazardous because of the radioisotopes incorporated into the probes. These arguments are now no longer valid since the introduction of biotin-labeled DNA probes (Langer *et al.*, 1981; Leary *et al.*, 1983; Brigati *et al.*, 1983; Myerson *et al.*, 1984). In principle any virus can be detected by this procedure, as long as it is feasible to obtain the viral genome in pure form. Figures 10–12 illustrate the methodology involved in probe

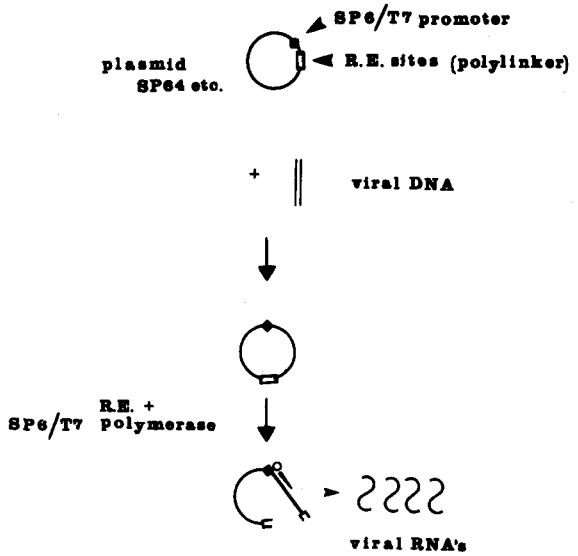

FIG. 11. Preparation and use of riboprobe system. R.E., restriction endonuclease.

production, specimen treatment and detection of the hybridized probe. If the virus has an RNA genome this can first be transcribed into a cDNA by means of reverse transcriptase (Hyypia *et al.*, 1984; Rotbart *et al.*, 1984). The routine production of adequate amounts of the viral DNA, or cDNA, can be achieved by propagation of the cloned genome or genome fragment in a plasmid or phage vector.

The versatility of the method should allow for the production of different family-specific, genus-specific or species-specific probes, as dictated by the portion of the viral genome used. For example, a fraction of the coxackievirus B3 viral genome is shared with other human enteroviruses, thus permitting a piece of cloned cDNA to be used as an enterovirus-probe (Hyypia *et al.*, 1984; Rotbart *et al.*, 1984). Other pieces of cDNAs could presumably serve roles as individual species-specific probes. Therefore it is possible to build up an armament of probes, which are quite stable when biotinylated, for covering the

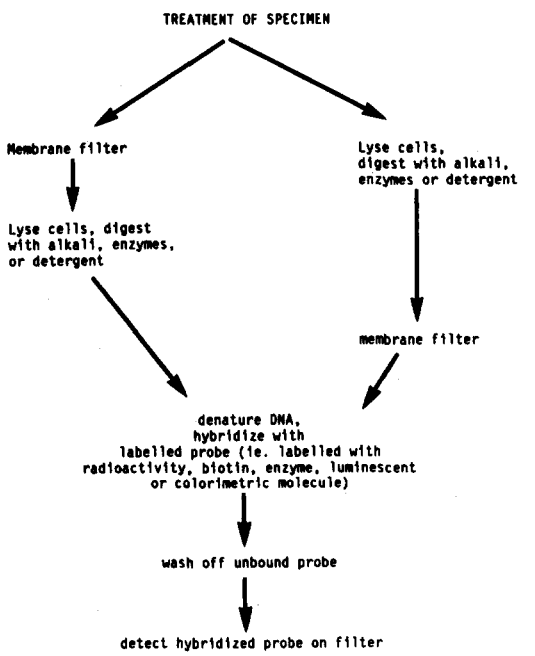

FIG. 12. Preparation of clinical specimens for hybridization with probe.

whole range of anticipated viruses, in much the same way that current practice calls for a battery of group-specific and species-specific antisera.

The development of a particular probe certainly costs money at the outset, but the technique should become cost effective when used routinely. Furthermore, the time element is rapidly disappearing as an obstacle, in view of the more recent experiences of investigators who can perform the complete test within one working day. The utility of the probe technique in the context of rapid viral diagnosis was recently discussed by Richman *et al.* (1984a,b).

The only remaining serious question concerns the ultimate sensitivity of the technique (Chou and Merigan, 1982; Virtanen *et al.*, 1983; Scotto *et al.*, 1983; Lieberman *et al.*, 1983; Anderson *et al.*, 1985; Clewley, 1985). At present several thousand virus particles in a specimen should be detectable, although it may require some further innovations to achieve the level of sensitivity already realized by cell culture isolation. It may not however be desirable to reach this level since, as already pointed out, the significance of only a few viruses in a specimen is open to doubt, especially if the probe detects defective or uninfectious virions which might be present in excess. Thus, one infectious particle detected in cell culture may in fact correspond to 10, or 100, or even 1000 total genome containing particles.

A particularly attractive feature of this approach is the realization of a diagnostic test for viruses which have presently defied attempts to cultivate them *in vitro*. Examples are the human hepatitis B virus (Scotto *et al.*, 1983; Lieberman *et al.*, 1983) and the human parvovirus associated with recurrent arthritic disease (Anderson *et al.*, 1985; Clewley, 1985). If similar prospects are realized for Veterinary viruses, this would circumvent the need for a multitude of different vertebrate cell lines.

The other outstanding problem, which should be relatively easy to surmount, is to facilitate the technical aspect of the procedure so that the entire operation can be carried out on multiple samples by a laboratory technician. One innovation in this regard is the development of improved methods of lysing the specimen, denaturing the DNA and its fixation onto nitrocellulose, all of which can be accomplished readily by means of exposure to chaotropic salts such as saturated NaI (Bresser *et al.*, 1983; Gillespie *et al.*, 1984).

In addition to biotinylated probes, fluorescent and enzyme-conjugated probes are being developed and these may have some advantages over the former (Landegent *et al.*, 1984; Renz and Kurz, 1984). In any case it is clear that a number of alternatives to radioactive probes are now possible and that these can be used with fixed tissue sections as well as fluid specimens.

Innovations will not stop here however, since the field of genetic engineering is expanding at a very fast rate. An example which may prove useful for diagnostic probes is the 'riboprobe' system, which has just become available.

Essentially this system comprises a plasmid (originally pBR322) which has been 'engineered' to include a specific bacterial phage promoter sequence (SP6) and an artificial sequence of nucleotides ('polylinker') containing multiple restriction endonuclease sites. In order to use this system, a piece of viral (or other) DNA of interest is cloned into this plasmid (referred to as pSP64 or pSP65) between the SP6 promoter and the polylinker. If the plasmid is then cut with a suitable restriction enzyme and incubated with the SP6 RNA polymerase, plus ribonucleotides, a faithful transcript (RNA) of the inserted foreign DNA results (Butler and Chamberlain, 1982). By including a radioactive ribonucleotide or biotin-UTP in the incubation mix, very high specific activity (or biotin-labeled) RNAs can be produced. These RNAs (even eukaryotic ones) can be translated efficiently *in vitro* and *in vivo* to give functional proteins (Green *et al.*, 1983; Krainer *et al.*, 1984; Zinn *et al.*, 1983; Maniatis *et al.*, 1982; Melton and Kreig, 1984). The RNAs can also be used as probes to detect corresponding nucleic acids by hybridization in liquid or *in situ* (Lynn *et al.*, 1983; DeLeon *et al.*, 1983; Cox *et al.*, 1984). A similar system has also been developed making use of the phage T7 promoter-polymerase, and is available commercially.

Although RNA is generally less attractive than DNA as a probe, because of its inherent instability at room temperature, this disadvantage may be overcome by the advantages

gained, which include high intensity of labeling the resultant probe and the greater sensitivity of DNA–RNA hybridization compared to DNA–DNA (Meinkoth and Wahl, 1984).

A different aspect of diagnosis is also amenable to newer techniques, namely the traditional serotyping, which helps to define the virus species and to record the epidemiology of a virus. The advent of monoclonal antibodies has made serotyping a more sophisticated tool, since many monoclonals may be derived for a single viral protein, each one recognizing a specific epitope on the virion (Nowinsky *et al.*, 1983). Direct testing can be performed with ELISA or similar tests. In addition the use of restriction-endonuclease 'fingerprinting' has already been described (Section 4) and illustrated for adenoviruses and for differentiation between related herpes viruses. It has also gained success in 'typing' HSV and HCMV strains (Lonsdale, 1979; Tyms, 1983). In an analogous fashion RNA genomes can be fingerprinted by the technique of oligonucleotide mapping of T_1-ribonuclease digests. This latter technique is capable of detecting one or a few nucleotide changes between two RNAs (Clewley and Bishop, 1982; Kew and Nottay, 1984).

These last few tests are not at present practical for a routine virology diagnostic laboratory, but they could be performed in a central reference laboratory. It seems likely that current and future strains of viruses will be catalogued to a much finer degree than has been accomplished in the past, notwithstanding the reservations already made about the significance of such detailed cataloguing. This type of analysis should aid the development of antiviral prophylaxis and therapy.

9. CONTROL OF VIRUS INFECTIONS

Control of virus infections, like any other kind of infection control, can be effected either as a prophylactic measure (i.e. before encountering the organism, in order to offer protection) or therapeutically, in order to control and alleviate a virus infection which has already been established in the host animal. There is no clear cut line of demarcation between these approaches, however, since successful control of an infection in an individual within a herd can prevent the infection spreading through the herd and therefore can be considered as a prophylactic measure.

Unlike most bacterial, fungal and parasitic infections however, viruses are not autonomous organisms and therefore require living cells in which to replicate. Consequently most of the steps in their replication involve normal cellular metabolic pathways, and this makes it difficult to design treatments to attack the virion directly, or its replication, without accompanying adverse effects on the cells. Fortunately some of the more complex viruses code for specific enzymes needed at some stage in replication, and these constitute potential targets. In fact most of the successful antiviral chemotherapy to date has been achieved with the herpes viruses because certain key viral enzymes have distinctive affinities for several nucleotide analogs (see below).

The subsequent discussion will concentrate firstly on vaccines, including several novel types of vaccine, and this will be followed by examination of current and prospective therapeutic approaches directed against the replicating virus and the infected cell.

9.1. Vaccines

Vaccination or immunization may be thought of as a means of preventing or ameliorating infectious disease by raising the level of immunity to an infectious organism or its toxins. Since toxins have not been reported for viruses then the prime consideration in this context is the infecting virus. Immunity is usually 'active' in that one or more of the viral antigens elicit immune responses. In some cases immunity is 'passive' as a result of deliberate transfer of serum globulins from an immune to a non-immune individual.

For the most part, consideration of vaccine efficacy has been limited to its ability to induce antibody formation, whereas in a natural infection it is recognized that cellular immunity is equally important and hopefully more attention in the future will be given to this aspect of vaccination.

9.1.1. *Killed and Live Virus Vaccines*

Traditionally vaccines were administered in clinical and veterinary practice in the form of chemically inactivated virus suspensions, although these were gradually replaced, when feasible, by suspensions of live viruses which were either attenuated forms of the corresponding wild virus or were less virulent relatives (Black, 1979, 1984; Norrby, 1983; Hilleman, 1984). Table 9 summarizes the principal features and the benefits and disadvantages of the two types. Clearly neither is ideal. In general live vaccines are better since they replicate within the host and thereby induce immune responses more efficiently. There are risks however, such as the possibility of reversion to a virulent form, and the problem of immunodeficient or immunocompromised animals. Doses of vaccine are calculated for healthy immunocompetent animals, and may consequently overload a deficient animal. In fact, experience with live vaccines in human medicine has illustrated rare complications.

Repeated use of inactivated virus usually results in successively less efficiency due to the phenomenon of 'original antigenic sin'. Thus the type of antibody produced in response to sequential vaccination reflects more and more the first strain historically encountered. It is this property which has enabled investigators to unravel the evolution of influenza A strains for the last 60 years or so.

In addition both types of vaccine result in persistence of the viral genome which, although it apparently does not provoke complications in practice(?), is nevertheless theoretically undesirable.

If the attenuation of a virus is the result of cumulative mutations, as is the case for the Sabin vaccine strain of polio virus type 1 (Baltimore, 1984; Nomoto *et al.*, 1984) or the deletion of a significant portion of essential genetic material, e.g. the pseudorabies attenuated vaccines (Lomniczi *et al.*, 1984a,b), then the vaccine may be thought of as 'crippled' and therefore unlikely to revert to the original wild form. In fact it should be possible to induce specifically designed mutations (e.g. by the technique of site-directed mutagenesis or by specific nuclease deletions of genetically engineered plasmid inserts). Such an approach is underway with polio virus vaccines (Baltimore, 1984) in order to ensure that a crippled virus vaccine cannot revert.

Vaccines are now used on a large scale for livestock throughout the world, although there is considerable variation between different countries regarding the type of vaccine used for specific virus infections. For example many vaccines are on the market in Europe for foot-and-mouth-disease and for pseudorabies, specific choices being dictated largely by past and current experiences with particular strains.

Some attempts have been made to produce recombinant whole-virus vaccines by selecting for the desired phenotypes following mixed infection of cell cultures by the related viruses (Chanock, 1981). For example recombining an avian influenza A strain with a

TABLE 9. *Disadvantages of Conventional Vaccines*

	Killed virus		Live virus
(i)	immunogenic mass small—consequently multiple doses required	(i)	virus replicates—one dose usually sufficient
(ii)	inadequate local immunity	(ii)	similar to wild virus
(iii)	small risk of residual virulent virus or contaminating viruses (assuming adequate quality control!)	(iii)	may be contaminated by virulent virus
(iv)	adverse effects of fixative		
(v)	viral genome persists, but in small amounts	(v)	viral genome amplified and persists
		(vi)	virus may revert to virulence if inadequately attenuated
		(vii)	may spread to susceptible contacts (could be beneficial or detrimental)
(viii)	wild virus may frequently change antigens—therefore protection incomplete	(viii)	same problem. Also over-attenuation may result in too much antigenic drift in vaccine
		(ix)	often labile in ambient environment—consequently shelf-life limited
(x)	examples: foot-and-mouth-disease; rabies; influenza	(x)	examples: yellow fever; influenza; rinderpest; turkey herpes (for Marek's disease)

human strain and selecting for the progeny viruses which have the human viral surface antigens, plus the restricted replication ability (in human cells) of the avian virus (Murphy *et al.*, 1982; Norrby, 1983). Other viruses with segmented genomes should be amenable to similar manipulation, although the concept has not really caught on.

Details on specific vaccines and their administration practices are beyond the scope of this review; they may be found in books and manuals on veterinary health practices. In general it is desirable to give a vaccine to a young animal, prior to contact with the wild virus, but only after its own immune system has matured. Since this time is different for different species (see Section 5) this means that a given vaccine is not given to all animal species at the same age.

9.1.2. *Subunit Vaccines*

In order to circumvent some of the problems associated with whole virus vaccines, attention has recently focused on the use of subviral preparations. In particular the rationale for this development is that since only the protein components of the virus are antigenic, there is no need to utilize the complete virion.

The first development in this approach made use of subunit vaccines, notably for influenza virus, which comprised detergent-disrupted virions from which the nucleic acids had been removed (Norrby, 1983).

Alternatively the principle antigenic determinant, in the case of influenza the HA (hemagglutinin) protein or the surface antigen of hepatitis B virus (Hilleman, 1984), may be used by itself. Such preparations avoid many of the theoretical objections raised to the use of whole virus preparations, but they are considerably more expensive because of the extra labor involved in producing them. Furthermore, individual soluble proteins usually require an adjuvant to stimulate an optimal immune response, whereas particulate preparations do not. A possible way around this drawback is to use micelles of virion envelopes, which, while retaining the major antigens, may also work without extra adjuvants. Such preparations have been made for influenza virus, bovine parainfluenza virus 3, and several other viruses at an experimental level (Almeida *et al.*, 1975; Morein *et al.*, 1978).

But for the reasons mentioned above subunit vaccines have not gained wide acceptance, especially for the routine vaccination of livestock. Their usefulness will probably be supplanted shortly by cloned viral proteins and synthetic peptides.

9.1.3. *Cloned Viral Proteins*

Since in most virus infections the immune responses, or at least antibody production, are directed principally against the one or two predominant virion proteins, preparations containing the latter only should suffice as vaccines provided that adequate adjuvant properties are also contained in the vaccines. Significant advances have been made recently in developing single protein vaccines for FMDV and for hepatitis B virus, by cloning the viral genes encoding these proteins into bacterial plasmids. Prospects look good for the production of similar vaccines for VSV and rabies. Consequently large scale cultivation of the bacteria can yield large quantities of the desired protein, which can then be purified and incorporated into a vaccine on an economically feasible level.

The FMDV particle is made up of four proteins, VP1, VP2, VP3 and VP4. The protein VP1 itself elicits the production of neutralizing antibody in animals. The first report of successful production of VP1 antigenic protein in bacteria appeared in 1981 (Kupper *et al.*, 1981). Figure 13 illustrates the principal steps involved in the cloning and expression of the VP1 gene in *E. coli*. The first step involved the synthesis of double-stranded cDNA (complementary DNA) molecules of most of the viral RNA genome (nearly 8000 nucleotides long), by means of reverse transciptase and *E. coli*. DNA polymerase. These cDNAs were used to generate restriction endonucleolease maps of the viral genome and to locate the gene for VP1. Suitable cDNAs were then cloned into plasmid pBR322 for

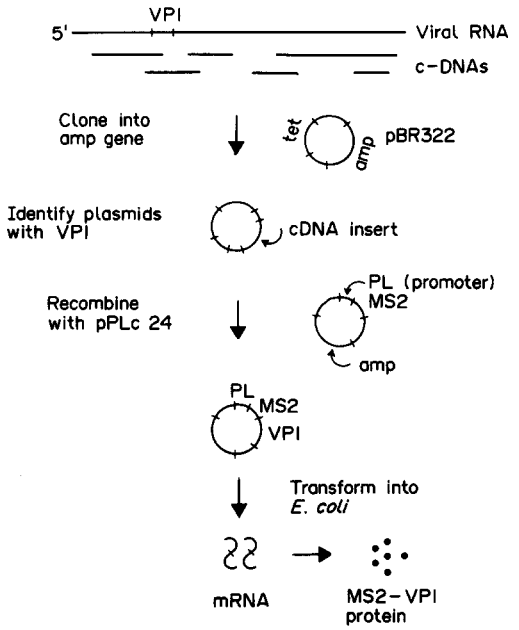

FIG. 13. Cloning and production of the major antigenic protein (VP1) of foot-and-mouth-disease virus. VP1, gene for VP1 protein. Plasmid pBR322 contains genes for resistance to tetracycline (tet) and ampicillin (amp), which permit selection of recombinant plasmids. Plasmid *p*PLc24 contains the PL-phage MS2 promoter system to allow efficient transcription of inserted (downstream) cDNA.

amplification and further characterization. Subsequently one of these plasmids, *p*FMDV-1034, containing the VP1 gene, was restricted to cut the VP1 coding region out of the plasmid vector. The excised piece was then ligated into a different plasmid, *p*PLc24, in such a way that the VP1 gene was now under the control of the strong lambda phage promoter provided by this plasmid vector. When the final reconstructed plasmid was introduced into an appropriate strain of *E. coli* the latter produced substantial amounts of VP1 protein fused to the plasmid promoter sequence plus some additional amino acids contributed by the plasmid vector. This polypeptide was able to induce FMDV-neutralizing antibodies in animals.

A similar procedure was reported by another group (Kleid *et al.*, 1981). In their case the fragment of cDNA containing most of the VP1 gene was incorporated into a plasmid containing a promoter–operator for the *E. coli* tryptophan genes. When this recombinant plasmid was introduced into *E. coli* and the cells were grown under conditions allowing optimal expression of the tryptophan system, a large hybrid protein was made which comprised tryptophan-specific protein plus the VP1 coded protein. This hybrid protein constituted 17% by weight of the total proteins synthesized by the bacteria, and it was able to elicit the production of FMDV-neutralizing antibodies in animals.

Essentially similar approaches were used to reconstruct plasmids containing appropriate genes for VSV and rabies virus (Rose and Shafferman, 1981; Yelverton *et al.*, 1983). These viruses are both rhabdoviruses, in which the principal antigenic groups are on the so-called G-protein, the glycosylated membrane protein. The final recombinant plasmids thus contained the VSV or rabies G-protein gene (or a large fraction of it) plus modified tryptophan promoter systems, which enabled the host *E. coli* to make substantial amounts of corresponding authentic protein. In the case of the rabies plasmid this amounted to up to 3% of total protein synthesis in the bacteria.

The key step in making this process commercially worthwhile is the incorporation of a strong promoter signal so that the foreign gene (e.g. VP1) introduced into the bacterial host will be expressed with a high frequency. In this way the protein of interest comprises a significant proportion of the total protein made by the bacteria.

In a further development the reconstructed FMDV-plasmid was recombined with yet another plasmid which allowed its replication and expression in *B. subtilis* (Hardy *et al.*, 1981), which offers some advantages over *E. coli* as a host for producing vaccines.

Whether or not this type of product can match the effectiveness or the cost of the killed virus vaccines in current use remains to be seen. For FMDV this may not be the case, since this virus is easily propagated in large quantities; but the conceptual approach outlined above can be applied to other viruses of veterinary concern, where a cloned product might turn out to be more practical than large scale virus production, e.g. rabies virus.

An additional problem inherent in the use of recombinant DNA in bacteria is that bacteria will not glycosylate proteins, or engage in other protein processing steps which might be essential to the maturation of antigenically active proteins. In the case of the examples cited this was evidently not a problem, but it was found to be so in the case of hepatitis B virus, where the bacterially-cloned protein was not glycosylated and consequently was not immunogenic in animals. This particular problem was subsequently circumvented by cloning the hepatitis B antigen in yeast cells, in which proper maturation of proteins can be expected (Valenzuela *et al.*, 1982; Hilleman, 1984).

An alternative method of ensuring proper processing and maturation of the viral protein is to use a eukaryotic vector which can then be propagated in mammalian cell lines. This was achieved for the influenza HA protein in a SV40 DNA vector (Sveda and Lai, 1981). Many other suitable vectors are available (Gluzman, 1982).

In spite of the pessimism which initially greeted the production of cloned viral protein as vaccines, it seems reasonable to conclude at present that there will be a place for such products in the veterinary vaccine market, at least for some viral diseases.

9.1.4. *Synthetic Peptides*

In view of the possible limitations in the use of cloned viral proteins, an alternative approach has been investigated, namely, to use only the antigenic part of the principal viral protein. In general the antigenic part (epitope) of the protein constitutes a small portion of the total amino-acid sequence. Therefore it should be possible to construct the appropriate peptide by chemical synthesis and to use this as the basis for a vaccine (Arnon, 1979; Sela and Arnon, 1982; Lerner, 1982; Sutcliffe *et al.*, 1983). Unfortunately peptides by themselves are only weakly immunogenic, and to offset this problem the peptide must be conjugated to a carrier protein. In addition it might still be necessary to incorporate an adjuvant into the vaccine, and this introduces a further complication since a given adjuvant does not necessarily work equally well in all species of animal.

Another valid objection is raised by the recognized fact that the antigenic region of a viral protein might be subject to considerable variation. This has been substantiated for several viruses, including FMDV and influenza A, in which the variation in such amino acid sequences is the cause of the varied serotypes of the virus. As a consequence of this variation existing killed or live virus vaccines must be continually modified in order to match the newly emerging viral strains. Peptide vaccines would not alleviate this complication.

In spite of these objections significant progress has been made in developing an immunogenic peptide which can protect animals from FMDV infection (Bittle *et al.*, 1982). In this procedure a series of peptides was synthesized, corresponding to specific regions of the VP1 protein. Of these, two were determined to have potent neutralizing activity against the virus *in vitro* when conjugated to carrier protein, and one, which contained amino acids 141–160 (total sequence 213 amino acids), was capable of protecting animals against a challenge by FMDV. This protection was afforded by a single inoculation of the peptide conjugate.

The efficacy of the conjugate was not as good as live virus vaccine, but was superior to the entire single protein VP1, presumably because maximal immunogenicity requires a specific conformation of the antigenic regions, and this is best attained in the intact virion, whereas the isolated complete protein VP1 does not achieve this conformation. On the

other hand conjugation of VP1 peptide to carrier protein allows partial restoration of the native conformation. Similar techniques have been used to develop influenza-A immunogenic peptides (Green *et al.*, 1982; Sutcliffe *et al.*, 1983; Shinnick *et al.*, 1984).

Thus the concept of synthetic peptides as vaccines looks promising in general (Beale, 1982), although at present it may not look too good in comparison with existing FMDV vaccines.

9.1.5. *Passive Immunization: Prospects for Monoclonal Antibodies*

Passive immunization, i.e. the administration of serum or gamma globulins, known to contain specific antiviral antibodies, to non-immune individuals, has been used for some time as an emergency measure to counteract critical virus infections. The process occurs naturally in most animal species when antibodies (usually globulins) are transferred across the placenta and in colostrum. However the term is usually restricted to therapeutic use in individuals who have acquired, or who have a high risk of acquiring, a potentially lethal infection, e.g. rabies, hepatitis B, and individuals who are immunosuppressed and have acquired virus infections such as chicken pox, measles (Hilleman, 1984).

Veterinary practice has been concerned mainly with emergency protection against rabies infection, where hyperimmune rabies antiserum is inoculated around the bite from a rabid animal (Baer, 1984; Koprowski, 1984). The problem here is that the donor of the antiserum is in general a different species from the victim, in which case anaphylactic shock can result.

In any event passive immunization is only intended as a temporary measure, since the antibodies are expected to decay in the recipient and so protection will only last for a few weeks or months. Nevertheless there may be a continual need for rabies antisera since this virus is still a scourge in many parts of the world and is persistently carried in wild populations (see Section 10). In the future some nations will have to decide whether to vaccinate all livestock and other domestic animals against rabies, as is done for FMDV in endemic areas, or instead to keep ample supplies of rabies antibodies for therapeutic use.

The adverse effects often elicited by heterologous antisera could be overcome if specific antiviral monoclonal antibodies were used. Extraneous serum components would be absent. Unfortunately prolonged use of heterologous monoclonals would probably lead to the generation of antibodies to the monoclonals themselves, but for short-term emergency use, as for example in rabies infection, it might be possible to administer an appropriate monoclonal derived from a mouse cell line. Since monoclonals are usually considerably more potent than hyperimmune sera, effective prophylaxis could probably be obtained by administering relatively little foreign protein.

9.1.6. *Viruses as Vectors: Supervaccines*

The practice of administering mixed viral vaccines is generally frowned upon because of the possible interference between two or more viruses, and because if one causes immunosuppression this might render the recipient animal unusually susceptible to the other virus. Alternatively an immune response might only be produced to one of them.

A possible way around this problem is to incorporate appropriate genes from two or more different viruses either into a liposome or into a large 'innocuous' virus. The drawback with the liposome approach (or any other synthetic carrier for that matter) is the difficulty in getting the liposomes to the right tissues, since they tend to migrate preferentially to the reticuloendothelial system where they are phagocytosed by macrophages (Gregoriades, 1980; Alving, 1983). In contrast a large virus (e.g. vaccinia or a similar pox virus) has the advantage of disseminating naturally. In this case one could incorporate several foreign viral genes into the virus, by recombinant DNA techniques, so that they would be amplified as a result of virus multiplication (i.e. like a live virus vaccine) and at the same time code for all the antigenic determinants of the parent viruses.

This approach does show some promise, and two groups of investigators successfully incorporated an influenza hemagglutinin gene (the principal antigenic determinant)

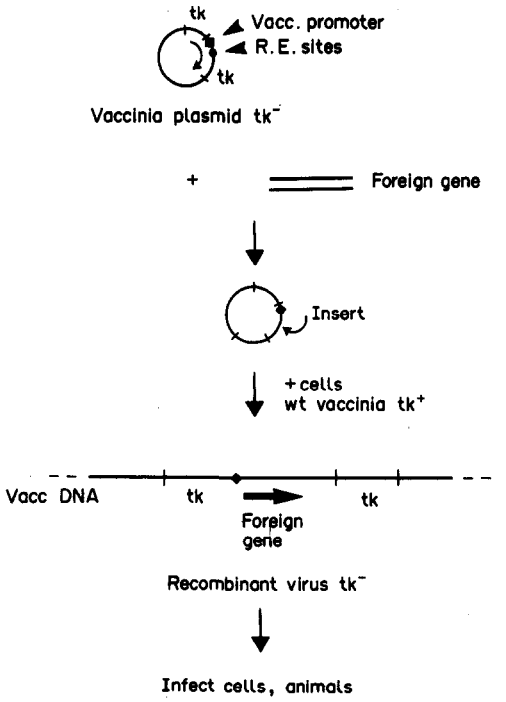

FIG. 14. Development of vaccinia recombinant virus. vacc, vaccinia; tk, thymidine kinase; wt, wild type; R.E. = restriction endonuclease.

into the vaccinia virus genome, with the result that the recombinant virus induced the production of influenza-neutralizing antibody in animals (Panicali *et al.*, 1983; Smith *et al.*, 1983a). In order to achieve this objective, special plasmids were constructed to contain the vaccinia thymidine kinase (TK) gene, certain control elements, the cluster of restriction endonuclease sites derived from a *p*UC plasmid, and the 'foreign' viral gene inserted into the TK region. The final chimeric plasmid was introduced into cells infected with wild type vaccinia virus, which then underwent some recombination events with the plasmid TK containing the insert such that the genuine desired recombinant vaccinia virions were devoid of TK activity. These recombinants were easily selected in TK^- cells (Mackett *et al.*, 1984). The Panicali procedure differs in detail but is essentially the same in principle (Panicali and Paoletti, 1982). The scheme is illustrated diagrammatically in Fig. 14.

More recently vaccinia recombinants have been produced containing genes for hepatitis B surface antigen (Smith and Moss, 1984; Smith *et al.*, 1983b); rabies virus glycoprotein (Kieny *et al.*, 1984); and herpes simplex virus glycoprotein D (Paoletti *et al.*, 1984; Smith and Moss, 1984). Presumably additional genes from other viruses could be inserted so that one could have a vaccinia virus which codes for several different viral antigens, i.e. a supervaccine.

The essential requirements for success in this approach are (i) the virus must have a large genome which can accommodate inserted foreign genes in place of its own non-essential genes; (ii) the virus itself should be relatively innocuous, yet should be able to replicate in many different animal species; and (iii) it should be possible to incorporate the foreign genes in such a way that they are governed by a suitable promoter–operator of the virus and consequently be expressed maximally. Presumably foreign signals could be incorporated if necessary. Although one would normally be hesitant in prescribing the deliberate inoculation of a particle containing an engineered viral genome, the vaccinia virus could reasonably be thought of as safe. This virus does fulfill the requirements listed above.

The standard vaccinia traditionally used for smallpox vaccinations was not without complications, a common finding noted by clinicians. But the virus could probably be attenuated further, or otherwise 'engineered', to alleviate the undesirable side effects.

Other eukaryotic viruses have been developed as vectors, e.g. papovaviruses such as polyoma, SV40, bovine papilloma virus type 1; retroviruses; defective herpes simplex virus

(Gluzman, 1982). But these have been designed mainly for research purposes, and at present their use as vaccines seems unlikely. They also have more restricted host ranges than vaccinia. However future 'engineering' of such vectors may eventually result in the development of completely innocuous yet efficient vaccines. For the time being the 'crippled' vaccinia virus concept is the only one which would meet safety standards.

The ultimate and logical extension of this concept is the generation of a 'plasmid' containing several foreign viral genes of interest together with one or more control elements and appropriate viral genes for permitting limited replication in specific tissues.

9.1.7. *Limitations in the Use of Vaccines*

Specific disadvantages of the different types of vaccine have been enumerated already in the preceding discussion. There are two other general features that should be considered. Firstly, the problem of genetic variation has been repeatedly emphasized. There is no reason to think that viruses will eventually stabilize; consequently vaccines of all kinds will continually have to be modified to accommodate 'antigenic drifts' and the occasional emergence of recombinant forms. In addition, although some viruses may occasionally be eradicated from human populations, as suggested by the recent success against smallpox, many other viruses probably await their turn to invade these interfering animals, as exemplified by the so-called exotic viruses which are usually innocuous in their natural hosts, and only gain recognition when humans stumble upon them accidentally. Similar events may also occur among non-human populations, especially when domesticated animals are introduced into new environments or when wild animals are displaced.

The second feature is the property of viruses to persist in wild populations, i.e. carriers or reservoirs. Smallpox could be eradicated because it was confined to humans. In contrast most of the devastating animal viruses usually persist in wild populations which are difficult to control. Thus clinicians in the USA are optimistic that mass vaccination against measles will result in virtual disappearance of this virus (Mitchell and Balfour, 1985), but obviously an analogous program would fail in the case of rabies or African swine fever, because these viruses would still persist in the wild reservoirs (see Section 10).

9.2. CHEMOTHERAPY

For many years virologists have sought chemical means of combatting virus infections. This has assumed greater importance in human medicine than it has in veterinary medicine, presumably reflecting the greater value placed on a human life than that of an individual animal. There are undoubtedly exceptions to this generalization, however, in situations where an important animal is infected, e.g. a racehorse, a prize bull, a domestic pet or a draught animal. In cases such as these it might be considered worthwhile to spend time and money injecting expensive drugs into the animal.

In spite of the intensive search for suitable antiviral chemicals relatively little success has ensued, although there is no doubt that many lives have been saved and numerous potentially crippling diseases have been averted through the use of judicious chemotherapy. One of the principal reasons for these successes is the fact that, while the virus itself may not be cleared from the body, the allevation of viral immunosuppression and histopathological effects are commendable goals, which can lessen the risk of secondary microbial infections and disease. Even relatively innocuous infections, such as herpes simplex genital sores in humans, are deemed worthy of appropriate chemotherapy because of their chronic discomfiture and risk of transmission. In animals the latter is the overriding feature, especially where there might be a chance of infecting a developing fetus or newborn.

In view of this it seems reasonable to continue the search for more potent, more specific antivirals, insofar as national research funding agencies permit. The chances of success in the future are significantly greater now that we appreciate the value of a rationalistic approach to the problem based upon more intimate knowledge of virus replication and its relationship to cellular metabolism.

TABLE 10. *Disadvantages of Chemotherapy*

	Feature	Examples
(i)	drug-resistant mutants *in vitro* and *in vivo*	IUdR, PAA, methisazone
(ii)	toxic side-effects	IUdR (if used systemically) Ara C; PAA; acyclovir
(iii)	conversion to ineffective compounds	
(iv)	compound may be mutagenic	IUdR, BUdR
(v)	immunosuppression *in vivo*	Ara C
(vi)	virus sensitivity may depend upon specific host cell	nucleotide analogs
(vii)	not all viruses within a family susceptible	CMVs often resistant to compounds which are active vs other herpes viruses
(viii)	some animal species may be more susceptible than others	

9.2.1. *Disadvantages of Antiviral Chemicals*

In general there are a number of important detracting features of chemotherapy, although they do not all apply to every chemical. These are listed in Table 10.

The subject of drug-resistance has been thoroughly reviewed by Loddo (1980). Viruses, like other organisms, frequently mutate to drug-resistant forms *in vitro* and *in vivo*, although the mechanisms vary for different chemicals. For example many of the nucleotide analogs require phosphorylation in order to interfere with viral replication, and this step is carried out by a thymidine kinase (TK). In herpes and pox virus-infected cells a viral-induced enzyme fulfills this function, especially when the host cells are non-dividing cells which have switched off the cellular TK enzyme. Mutants frequently arise which are defective in TK; consequently the compound cannot be phosphorylated, at least to its normal level, and so the virus escapes inhibition. There is evidence that such TK defective mutants are less pathogenic than their wild counterparts; but although this may be a common feature of some mutant viruses (i.e. they may be somewhat attenuated), it may not be universal.

Phosphonoacetate and phorphonoformate react directly with herpes virus DNA polymerases, thereby inhibiting virus replication. However, resistant mutants frequently arise in tissue cultures and in animal models as a result of which the enzyme functions in the presence of the compounds (Gauri, 1981). From the mutant populations revertants (i.e. sensitive mutants) occasionally emerge and in these the enzyme is once again inhibited.

This property emphasizes the inherent genetic instability of viruses and enforces caution in the widespread use of antiviral chemicals. The experience of antibiotic-resistant bacteria has taught us a useful lesson in this respect. But on the other hand we should also realize that the purpose of chemotherapy in the veterinary field is for short-term emergency use, and it may be that the occasional mutant which inadvertently is selected in a short time period is not significant. In terms of the analogy with antibiotics, these compounds have an important role to play in human and veterinary medicine in spite of the potential for drug-resistant mutants.

Another interesting phenomenon is the presence of drug-dependent viral mutants, which have been found to arise frequently among enteroviruses and some pox viruses treated with certain chemicals. Such mutants thrive in the presence of excessive concentrations of the antiviral, where they obviously have a selective advantage, but they grow poorly in the absence of the chemical (Loddo, 1980). It is not known if this phenomenon has any significance *in vivo*, but in any case it would probably only present a problem after prolonged therapy.

Adverse side-effects often accompany the use of novel agents in animals and these are usually not predicted from the preliminary tissue culture trials. Most of the antivirals tested to date can have some detrimental effect upon host cells and tissues when administered in doses beyond their minimal inhibitory levels, but in some cases these can be tolerated in the interests of alleviating a serious infection. Additional side-effects, however, may become evident following chronic or repeated use of a compound.

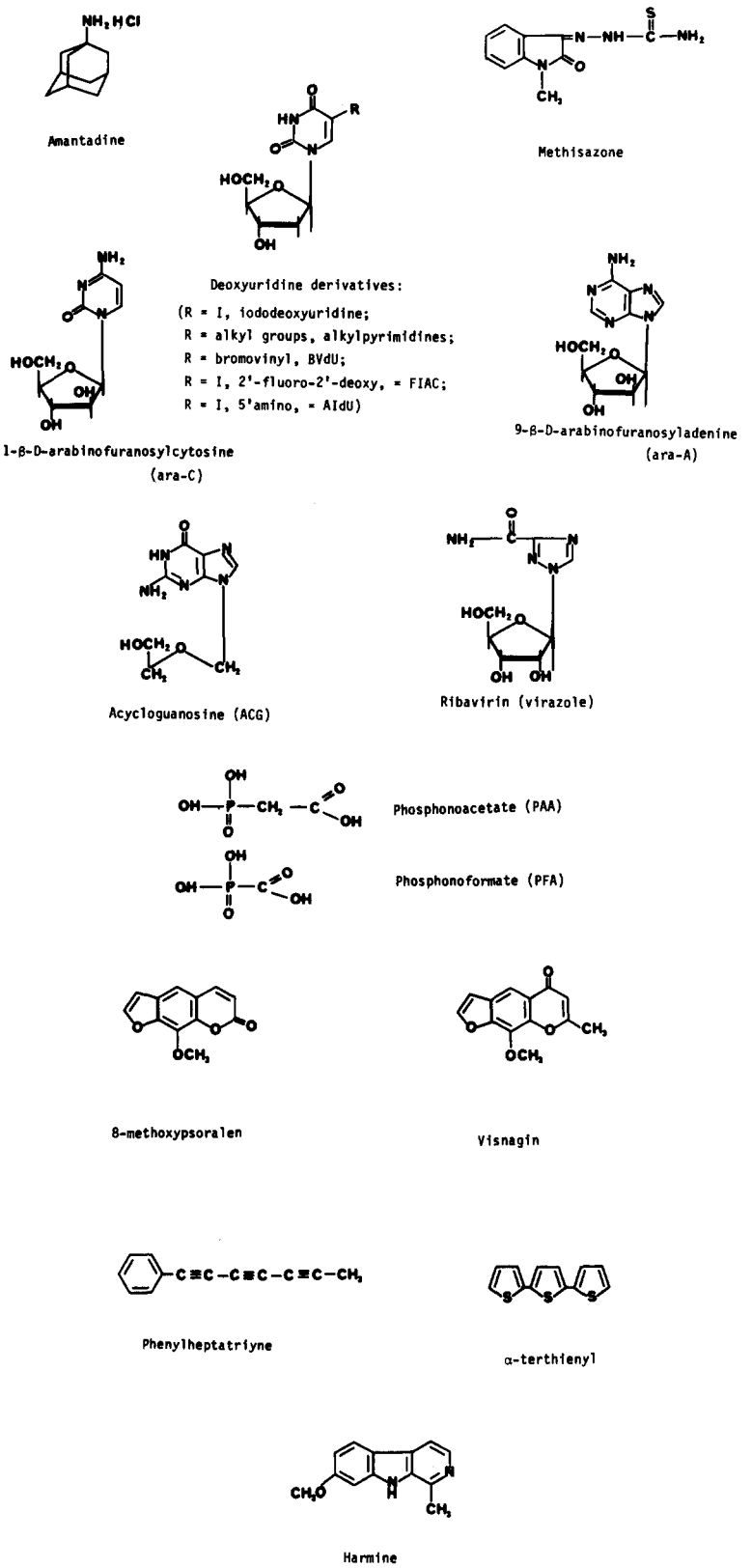

FIG. 15. Structural formulae of antiviral chemicals discussed in text.

Some of the compounds are immunosuppressive by virtue of their effects upon leukocytes and their precursors, but again short-term application may not cause problems.

Appropriate restrictions in the dose and mode of application can turn potentially dangerous chemicals into useful ones, e.g. IUdR, which is toxic when used systemically but is effective against superficial herpes infections (Cohen, 1979; Pavan-Langston, 1984).

In addition to these factors, it is also clear that different cells and tissues, and different species of animal, respond in different ways to a given antiviral. For this reason it is vital that potential agents are adequately tested before being marketed.

The next few subsections describe briefly the major antiviral chemicals which have found some use in controlling virus infections *in vivo*. The formulae for these compounds are presented in Fig. 15.

9.2.2. *Amantadine*

Amantadine (adamantine) hydrochloride has been known for more than 20 years as an effective agent in controlling influenza A infections (Oxford and Galbraith, 1980, 1984). In cell cultures it is capable of preventing the replication of this virus and several other RNA viruses. Its mechanism of action is not understood completely, although in the case of influenza A virus it is clear that an early event in the replication cycle is inhibited. Thus, the adsorption and penetration of the virus into cells proceeds normally but early transcription does not commence. It has been inferred from these results that the uncoating of the virion is blocked (Skehel *et al.*, 1978). However, before the viral genome can initiate transcription it has to migrate into the cell nucleus, consequently there are a number of potential target sites for antiviral attack apart from the uncoating events (Hudson *et al.*, 1978; Dimmock, 1982; Herz *et al.*, 1981).

More recently we have found that one of the fish rhabdoviruses, infectious hematopoietic necrosis virus (IHNV) is also inhibited in cell cultures (unpublished data).

Unfortunately only influenza A virus is apparently affected by amantadine *in vivo*. Swine 'flu, i.e. the influenza A strain isolated from swine, is also controlled *in vivo* (Oxford and Galbraith, 1980, 1984). The attractive feature of the compound from the veterinary aspect is the observation that it can be mixed in with the feed. For example influenza infection was successfully controlled in turkeys in this manner (Lang *et al.*, 1970; McGahen *et al.*, 1970). It would seem worthwhile testing its usefulness in other animal populations, including fish, e.g. for rhabdovirus infections in salmonids, by likewise incorporating it into suitable feed pellets.

In humans the compound has been found effective in controlling the spread of influenza A in clinical trials and during a natural infection, and for this purpose the drug is licensed, but its use has not gained wide acceptance. Adverse side-effects have apparently been minimal.

More details about amantadine and its derivatives, such as the more potent rimantadine, have been presented in the very comprehensive review by Oxford and Galbraith (1984).

9.2.3. *Methisazone (IBT)*

Methisazone is the other well-tried and established antiviral agent, which has a history somewhat analogous to amantadine. It specifically inhibits the replication of some pox viruses *in vitro* and *in vivo* (Bauer, 1965). It has been found to be effective in certain clinical situations (McLean, 1977).

The compound apparently interferes with the translation of late viral proteins, with the result that progeny virions are not formed, although uninfected cells are not affected. The compound, or its derivatives, have also been found to work effectively in inhibiting some adenoviruses and enteroviruses in cell culture systems. Nevertheless it does not appear to have found application in veterinary practice.

9.2.4. *Arabinosides*

The two agents in this category which have undergone numerous animal and clinical studies are the adenine and cytosine arabinosides, or Ara A and Ara C respectively. The thymidine analog Ara T has received less attention (Prusoff *et al.*, 1981; North and Cohen, 1984).

Cytosine arabinoside initially appeared to be more satisfactory in terms of antiviral effects in the cell cultures, being effective against several DNA-virus groups. It was less satisfactory *in vivo*, however, because of various adverse side-effects, and although it was found to decrease mortality in cases of herpes encephalitis, its use has been discontinued (Cohen, 1979; Shannon, 1984).

Adenine arabinoside (vidarabine) was eventually licensed for use in clinical medicine, where it has since found use in the treatment of herpes encephalitis. Initial disadvantages of the compound were its low solubility, which necessitated the infusion of large volumes into the patient, and its rapid metabolism (Shugar, 1980; North and Cohen, 1984; Shannon, 1984). The solubility problem was circumvented by switching to the monophosphate derivative Ara AMP. This compound is in any case less toxic and immunosuppressive than Ara A, although because of its charged nature it penetrates the cell membrane relatively poorly. The application of Ara AMP plus iontophoresis (i.e. an electric current which enhances permeability of cells to charged compounds) increases the efficacy of Ara AMP in the treatment of cutaneous herpes infections (Hill *et al.*, 1977). A further development was the use of an Ara ester which has an increased solubility and penetrability and which is hydrolysed in tissue to Ara A (Shannon, 1984).

The second problem associated with Ara A, rapid metabolism, is due mainly to the presence, in some cells, of large amounts of adenosine deaminase, which removes the amino group from the purine. This has been tackled by the simultaneous application of an inhibitor of the enzyme, thus prolonging the half-life of the active component. For this purpose the commercially available therapy was made available comprising 'vidarabine and covidarabine'.

The mechanism of action of the arabinosides requires conversion to their corresponding triphosphates, by cellular enzymes. The triphosphates are incorporated into cell and viral DNA and inhibit further DNA polymerase action. Their relative selectivity towards herpes replication reflects the greater sensitivity of the viral DNA-polymerase, although most cellular and other viral polymerases are inhibited by higher concentrations of the Ara triphosphates. It should be noted that herpes viruses which do not code for TK enzymes, namely cytomegaloviruses which are relatively resistant to certain other nucleotide derivatives, should be sensitive to arabinosides. This is in fact the case.

9.2.5. *Pyrimidine Nucleoside Analogs*

Iododeoxyuridine (IUdR) was the first of this group of compounds to be used in animal models, and clinically, to control herpes infections. It is too toxic to consider for systemic application, but it is still frequently used for topical application in herpes keratitis and cold sores, where relief is often obtained (Pavan-Langston, 1984; Prusoff *et al.*, 1984). Lesions sometimes heal faster in the presence of IUdR.

Unfortunately the compound does not discriminate between viral and cell DNA synthesis, with the result that uninfected dividing cells are easily killed.

More recently a large group of pyrimidine nucleoside analogs has been designed to inhibit specifically the viral enzymes without adverse effects on the corresponding cellular enzymes. This major advance heralds what Prusoff has described as the phase of 'serendipitous specificity' in antiviral therapy (Prusoff *et al.*, 1981; De Clercq, 1984). Since it is now clear that herpes virus TK (in reality a deoxypyrimidine kinase; not just a thymidine kinase, Gentry *et al.*, 1981) and herpes virus DNA polymerases have quite different properties from the cellular enzymes it has become feasible to search for, or to synthesize, chemicals which are either selectively phosphorylated by the viral TK or which selectively inhibit viral DNA polymerase.

Analogous arguments can be applied to other viruses which code for their own enzymes, but to date most attention has focused on herpes viruses.

The most notable compounds in this category are the 5-substituted deoxyuridines and deoxycytidines, AIdUrd, FIAC, and BVdU (see structures in Fig. 15) (Walker *et al.*, 1979; De Clerq, 1979, 1984; Shugar, 1980; Shannon, 1984). These are all preferentially phosphorylated by herpes TK enzymes and the triphosphates consequently compete with the normal ones. In addition some of the triphosphates are much more effective in inhibiting the herpes DNA polymerase than cellular polymerases. However the principal reason for their very high therapeutic ratio, i.e. low toxicity is the fact that uninfected cells cannot significantly phosphorylate these compounds.

It should be noted however, that not all herpes viruses are equally sensitive and in fact it could be predicted that cytomegaloviruses, which do not code for TK-like enzymes (Muller and Hudson, 1977b), and other viruses which code for TKs with more restricted substrate specificity, would be relatively insensitive.

Nevertheless this does represent an important improvement in the development of antiviral drugs, because the way is now open for deliberate design and synthesis of nucleoside derivatives with desired modifications.

A point worthy of consideration is that it is not always possible to extrapolate directly from a given test sytem to an animal model or the clinical situation. Different compounds may be metabolized differently by different cells, tissues or animals. For example the deoxycytidine deaminase can deaminate some pyrimidine nucleoside derivatives and consequently might render them refractory to phosphorylation. The level of this enzyme varies tremendously, from very low amounts in mouse and rat cells to large amounts in human and hamster cells. Similarly cell lines vary, e.g. BHK cells have very little; Hep-2 cells have lots (Gentry *et al.*, 1981). Also many enzymes involved in nucleic acid metabolism fluctuate with cell-cycle status (Weissbach, 1975; Baserga, 1976). This kind of variation probably helps to explain some of the apparent discrepancies among different reports.

Other pyrimidine derivatives with potential antiherpes activity have been found, but these are limited by virtue of low penetration into cells (e.g. alkylpyrimidines) (Bilello *et al.*, 1981). However, it turns out that some of these compounds can readily penetrate herpes-infected cells, which are often more permeable than uninfected cells (Koch *et al.*, 1981). This affords us another mode of selective attack against the virus. As explained elsewhere in this review, it is probably a good idea to kill the infected cell, rather than just to inhibit virus replication, in order to decrease the likelihood of a latent infection.

9.2.6. *Acycloguanosine*

This compound, ACG (otherwise known as acyclovir-ACV) has been very successful in the treatment of herpes simplex infections in various animals. Like the pyrimidine nucleosides, ACG owes its high therapeutic index to the fact that it is poorly phosphorylated, if at all, in uninfected cells. By contrast HSV-TK efficiently phosphorylates it, whereupon cellular enzymes produce the triphosphate, which is incorporated into DNA by DNA polymerases. Since the compound contains an acyclic sugar, this effectively blocks elongation of the newly synthesized DNA strand (Shugar, 1980; Ericksson *et al.*, 1981; Field *et al.*, 1981; Shannon, 1984). It should be noted that infected cells containing large thymidine pools, which are expected to compete for TK-phosphorylation, are less sensitive to ACG inhibition than cells with small pools of thymidine.

The requirement for a herpes TK was shown by the introduction of the HSV-TK gene, via a plasmid, into uninfected cells. When ACG was added to these 'TK-transformed' cells, the ACG was phosphorylated and consequently inhibited DNA synthesis (Crumpacker *et al.*, 1981).

HSV mutants resistant to ACG have been shown to code for a TK with altered substrate specificity, so that ACG is not phosphorylated; or they have a resistant DNA polymerase (Crumpacker *et al.*, 1981). Contrary to expectation ACG is effective *in vitro* and *in vivo*

against murine CMV (Burns *et al.*, 1981). This virus does not code for a TK enzyme (Muller and Hudson, 1977b), so presumably another viral enzyme is able to phosphorylate ACG (but does not phosphorylate TdR), or the mechanism of action is different in this case.

In the clinical situation, ACG has proven useful both in topical treatment of herpes lesions of various kinds, and systemically against disseminated herpes simplex and herpes zoster (shingles). Long term use has currently fallen out of favor in view of its potential for renal toxicity. Nevertheless since no antiviral compound has ever been promoted for the purpose of prolonged therapy this should not be taken as a strike against ACG.

Other acyclo-analogs of purines and pyrimidines are also undergoing evaluation, with the intention of improving the therapeutic index even further (Shannon, 1984).

9.2.7. *Phosphonoacetate and Phosphonoformate (Foscarnet)*

These pyrophosphate analogs. PAA and PFA respectively, have been investigated for several years as antiherpes drugs. PAA is active only against herpes viruses, and in any case is not useful for *in vivo* application because of adverse side-effects. PFA is more successful in this regard and is also active (at least *in vitro*) against several other viruses which possess polymerases viz. hepatitis B, retroviruses and influenza A (Helgstrand *et al.*, 1981; Shannon, 1984).

These compounds penetrate cells readily and inhibit DNA polymerases, apparently by competitively binding to the pyrophosphate binding site of the enzyme. Evidently the herpes simplex enzyme is substantially more sensitive than the cellular polymerases.

A variety of other analogs and derivatives of PFA have been synthesized, but none is as effective as PFA itself. PFA is considered more effective in treating cutaneous HSV lesions than ACG in animal models, and is also clinically useful for cold sores.

9.2.8. *Other Compounds*

Numerous studies are underway with various other nucleoside derivatives, using herpes simplex and herpes viruses of veterinary interest as targets (Buchanan *et al.*, 1984; De Clerq, 1979). Pseudorabies virus and equine herpes viruses are now popular, and presumably some of these compounds and viruses will be used in large animal studies soon.

Meanwhile the search continues for chemicals with a broader spectrum of antiviral activity. A synthetic compound which has shown promise in this regard is ribavirin (virazole; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide). This compound structurally resembles guanosine and inosine nucleotides and accordingly competitively interferes with the key steps in the synthesis of GTP and dGTP in cells. Ribavirin is phosphorylated by adenosine kinase and the resulting monophosphate competitively inhibits IMP dehydrogenase, effectively blocking guanosine and deoxyguanosine triphosphate formation. This would explain the action of ribavirin in decreasing DNA and RNA synthesis in mammalian cells (Muller, 1979; Shugar, 1980; Shannon, 1984). But in order to explain the selective antiviral effects, which are expressed at much lower ribavirin concentrations, additional mechanisms must operate.

Ribavirin exists principally in treated cells as the 5′-triphosphate, but very little of it is incorporated into DNA and RNA. However some viral polymerases are sensitive to this triphosphate, notably influenza RNA polymerase and HSV-1 and -2 DNA polymerases. In contrast cellular DNA and RNA-polymerases are resistant.

This offers a possible mechanism to explain part of the antiviral spectrum, though not all. Results do vary significantly in different cell culture models. An additional mechanism is available for some viruses, e.g. pox viruses, where ribavirin interferes with the GMP incorporation step in the capping of mRNA (Shannon, 1984). However, there are still many viruses which are quite resistant to the compound, some of which might be expected to present potential targets, e.g. the rhabdoviruses which carry their own virion RNA polymerases, and pseudorabies virus which, like HSV, codes for a viral DNA polymerase.

Also picornaviruses and togaviruses tend to be relatively resistant. All of these observations together underscore the dilemma still facing investigators today, viz. the empirical nature of the antiviral testing programs.

In view of the high therapeutic index of ribavirin to many viruses, it was no surprise to find that the compound could be administered successfully to various animal models of viral diseases, topically, orally, and systemically, without adverse side-effects.

Unfortunately less success has been achieved in clinical situations. While ribavirin has been reported to control various types of cutaneous herpes infection effectively (and apparently herpes zoster following systemic treatment), as well as some acute cases of hepatitis A or B, it is much less effective against influenza. Nevertheless since ribavirin has such attractive features it would seem worthwhile to try in therapy of selected veterinary infections.

A group of beta-diketones, particularly arildone, were enthusiastically greeted when they became available because of their apparent broad antiviral spectrum coupled with fairly high therapeutic index. But they have not been tested in many animal model and clinical situations (Diana *et al.*, 1977; Kim *et al.*, 1980; Tyms *et al.*, 1984).

A compound worth mentioning because of its success in controlling Marek's disease in chickens is impacarzinum (DPT1, an imidazolidone derivative). This compound was found to inhibit turkey herpes virus (HVT) replication in cell cultures (Kaaden and Neth, 1981). It was subsequently administered to chickens in drinking water and thus significantly reduced tumor formation and mortality from MDV. The mechanism of action is not clear however since it did not prevent the dissemination of the MDV in the infected chickens nor the production of anti-MDV antibodies. Perhaps this compound works indirectly *in vivo*, e.g. as a stimulator of the reticuloendothelial system or interferon production. The fact that it works in a relevant animal model would seem to justify continued investigation.

Many other chemicals, synthetic and natural have been tested as antivirals in cell culture models and in some cases in animal models, but there seems to have been little systematic attempt to apply any of these to domestic animals or to fish. More details on the naturally-occurring chemicals can be found in the review by Becker (1980).

Thus, the prospects for chemotherapy of a few specific virus infections, notably herpes infections, are good in view of our ability to design chemicals for the specific task of interfering with viral enzymes. Similar approaches may be fruitful for other groups of viruses, e.g. the current attempts to find non-toxic chemicals which interfere specifically in the viral enzyme-directed processing of precursor proteins in the picornaviruses. Blockage of this stage results in the unavailability of functional viral proteins and hence virus replication cannot proceed. But for the majority of viruses, testing is still largely a trial-and-error situation. A brief review of the problem from the standpoint of the veterinarian has been presented by Gustafson (1980). The related subject of methods of drug delivery to animals, and the pharmacology of such chemicals in healthy and diseased animals, are important features but are outside the scope of this review. Useful articles on these subjects have been written (e.g. Pitman and Rostas, 1981; Baggot, 1980).

In the veterinary field it may well be that the concept of antiviral chemotherapy is abandoned in favor of a more rational approach which attempts to improve or induce the resistance of the host, e.g. through the use of immune modulators (Werner and Zerial, 1984), vitamin therapy etc. (Siegel and Morton, 1977).

9.3. Photochemotherapy

The term photochemotherapy usually conjures up a picture of the application of the light sensitive dyes, such as neutral-red and proflavine, to the treatment of topical herpes simplex infections. This form of therapy, otherwise referred to as photodynamic therapy, was used in many cases to treat cold sores, genital sores and keratitis, with some success. The subject was reviewed recently from the basic and clinical aspects (Bockstahler *et al.*, 1979, 1984; Wolff and Honigsmann, 1981).

The principle of the treatment depends upon the ability of the dye to intercalate between neighboring nucleotides in the DNA (or RNA), or to bind electrostatically to the

nucleotide bases. Upon exposure to light of the appropriate wavelength, usually in the visible or near-u.v. range, damage to the DNA results in the form of single- or double-strand breaks. This process takes place in both viral and cellular DNA, so that viral replication is inhibited. Probably the infected cell, and adjacent uninfected cells as well, will die as a consequence. Since cell turnover is relatively rapid in areas afflicted by the herpes simplex lesions, then the lesion should heal quickly after the dead cells have been sloughed off (Hollenberg *et al.*, 1976).

Other macromolecular components of the cell may be adversely affected by the treatment, but the principal damage is thought to involve the nucleic acids.

In addition to the obvious potential for side-effects resulting from damage to cell components, the spectre of treatment-induced oncogenesis has been raised. Herpes viruses all seem to possess cell-transforming capacity, especially when their ability to replicate and to kill cells has been inactivated by various means. In fact Rapp and colleagues have shown that photo-inactivated herpes simplex viruses can transform animal cells, and that some of these transformed cells can produce tumors in animals (Duff and Rapp, 1973; Rapp *et al.*, 1973). In addition the dyes are themselves mutagenic, and thus may give rise to undesirable herpes virus mutants, or deleterious mutations in the cells (Lochmann and Micheler, 1979; Bockstahler *et al.*, 1984). For these reasons photodynamic dye therapy has waned in interest and is no longer considered as a safe method of treating virus infections.

Recently there has been renewed interest in phototherapy because of the realization that there are numerous naturally occurring plant substances, many of which have anti-microbial activity, which are active in the presence of light. Table 11 summarizes their properties and shows a few examples (see also Fig. 15 for formulae). Some of these compounds are already familiar to clinical practice, e.g. the psoralens, which have been used for the treatment of psoriasis and other skin disorders. Many of them constitute the 'active principles' of plants which have had a history of successful treatment of skin affliction, including microbial infections (Spikes, 1975; Towers, 1979, 1984; Towers, 1984; Poulton and Ashwood-Smith, 1983).

Unfortunately some of these 'photosensitizers' do not discriminate between viral and cellular macromolecules, but others look more promising. In this respect their spectrum of activity is greater than just herpes viruses. A systematic study of the antiviral properties of these compounds has not been reported, although it would seem worthwhile. As a corollary to this statement it may be mentioned that most animals, domestic and wild, do come in contact with and consume many of these photosensitizers in the form of their normal intake of foliage. This is of course particularly relevant to the herbivores, which do comprise the majority of our livestock.

More detailed examination of the antiviral properties of these photosensitizers seems justifiable, and we have undertaken to analyze them further (e.g. Hudson *et al.*, 1982, 1985, 1986a,b).

9.4. MONOCLONAL-ANTIBODY CONJUGATES

Chemotherapy against virus infections would be made more attractive if it were possible to target the chemical selectively to the infected cells, thereby killing them whilst sparing the neighboring uninfected cells.

TABLE 11. *Antiviral Photosensitizers**

Compound	Type	Phototoxic to viruses: With membranes	Phototoxic to viruses: Without membranes	Apparent primary target(s)
Phenylheptatriyne	polyacetylene	+	−	membrane proteins?
α-Terthienyl	thiophene	+	±	membrane fatty acids (proteins)
8-methoxypsoralen	furanocoumarin	+	+	DNA, RNA
Visnagin	furanochromone	+	+	DNA, RNA
Harmine	β-carboline alkaloid	+	+	uncertain, possibly DNA, RNA, proteins

*For further details, see Towers, 1984; Hudson *et al.*, 1982, 1985, 1986a,b.

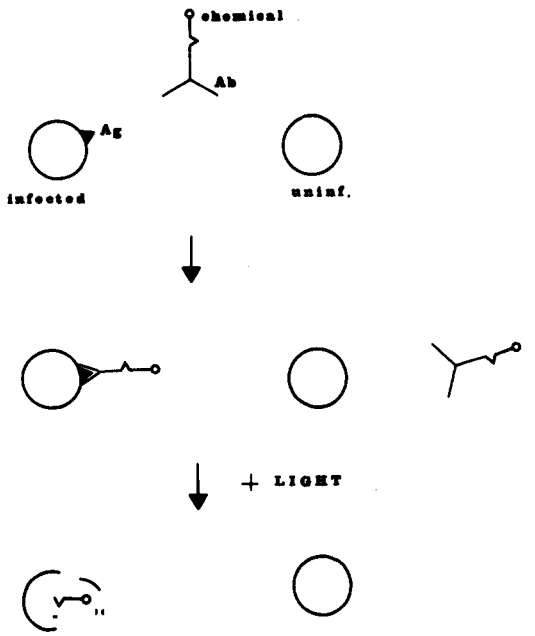

FIG. 16. Mechanism of action of monoclonal-antibody photochemical conjugate. Photoactive compound is chemically linked to monoclonal antibody (Ab) which is specific for the antigen (Ag) expressed on the infected cell. The uninfected cell survives the treatment.

In principle this approach, which is illustrated in Fig. 16, is feasible for any infected cell which expresses viral antigens on the cell surface. The monoclonal antibody component of a chemical conjugate would selectively bind to appropriate viral antigens and subsequently become internalized, whereupon the attached chemical could then cause its damage (Edwards, 1983; Magee and Ristow, 1983).

In practice more than half of the viruses encountered by animals have membranes and code for one or more viral proteins which are inserted into the cell membrane during replication. The insertion tends to occur at discrete sites of the membrane, known as 'patches', which consist of clusters of antigens easily recognized by antiviral antibodies.

The rationale for this approach was first developed for application to chemotherapy of experimental cancer. The earlier chemicals used included some potentially toxic substances, which were apparently without effect on the host cells or animals by virtue of their irreversible linkage to the antibody molecule; consequently only those cells expressing tumor-antigens complementary to the antibody were destroyed (Gilliland *et al.*, 1980; Vitetta *et al.*, 1983).

Nevertheless the use of potentially dangerous substances in therapy is frowned upon. In order to circumvent this problem Towers and colleagues extended the technique by conjugating the tumor-specific monoclonal-antibody to a light-sensitive compound which itself has clinical application, namely hematoporphyrin (Spikes, 1975; Dougherty *et al.*, 1978; Mew *et al.*, 1983). When this conjugate was used it was found that tumor cells in a mouse would bind the conjugate, but that exposure of the animals to intense white light was needed to activate the porphyrin moiety and hence to kill the tumor cells. Thus the combination of a photosensitizer and the antibody provided two important advantages: (i) the desired specificity of the reaction; and (ii) the innocuous nature of the conjugate in the absence of light activation. The term 'photoimmunotherapy' was coined to describe this technique (Mew *et al.*, 1983).

In theory the technique should be applicable to virus-infected animals. There are however two limitations. Firstly the question of light penetration arises in connection with large animals. Although visible radiation, especially at the red end of the spectrum, exhibits a surprisingly high degree of penetration in tissues, its use is still only limited to the external several millimeters of exposed skin. This was evidently adequate in the case of the mice

referred to above, but would not be too useful in a large thick-skinned bovine. A solution to this problem would be the use of optical fibres which can be designed today to reach almost any tissue in the body.

The second limitation is the timing of the treatment. The infected cells only express surface viral antigens at a certain time in the replication cycle and therefore are probably only accessible to the conjugate for a limited period of time. But in practice this might not be a real problem since infection within a tissue is bound to be asynchronous. Consequently the conjugate, which presumably will circulate in the animal for some time before being disposed of, will frequently encounter infected cells ripe for attack.

A tremendous advantage of this kind of treatment, compared with conventional chemotherapy, is the possibility of destroying persistently-infected cells. As long as the cells harboring a latent viral genome or a chronic virus infection express surface antigens, which they sometimes do, then they are amenable to attack.

This entire concept is still largely theoretical at this time, although it is undergoing experimental testing. Any of the photosensitizers mentioned previously afford potential tools for exploiting this approach, as do the myriad of other antibiotics and antiviral compounds presently being used alone for therapy. The chemical techniques for conjugating most of these compounds to antibody molecules have been worked out (see e.g. the review by Edwards, 1983).

9.5. Vehicles for Delivery of Compounds

Various particulate materials have been advocated as possible vehicles or carriers of chemicals or macromolecules to tissues in the body, in order to prevent destruction of the compound by enzymes, chemical attack, or sequestration. Among the vehicles proposed are such things as liposomes, erythrocyte ghosts, plastic beads, polynucleotides etc. (reviewed by Gregoriades, 1980).

Liposomes have received most attention because of their flexibility and relative reproducibility. In this approach a selected compound would be incorporated into the appropriate type of liposome and inoculated into an infected animal. Within individual cells the liposomes would be broken down by lysosomal enzymes and their contents released. Unfortunately there are two key disadvantages to this approach: (i) the lack of specificity, since infected and uninfected cells would both receive the same dose of compound; (ii) the lack of control of the liposomes, which tend to migrate preferentially to reticuloendothelial tissues (Gregoriadis *et al.*, 1977). This feature of liposomes may be useful for combatting micro-organisms which inhabit macrophages, e.g. *Leishmania* (Alving, 1983), but is not useful for the majority of virus infections.

The same criticisms can be leveled at the other forms of carriers or vehicles. Thus, unless some modification can be made to the vehicle, in order to permit control of its destination, this approach seems to offer little hope of success to the veterinary virologist.

9.6. Interferons

Section 5.5 reviewed the current status of IFNs and their apparent mechanisms of action against viruses. In regard to their clinical and veterinary use, relatively little success has been achieved over the years, either as a prophylactic or as a therapeutic measure (see the review by Scott, 1983). The optimistic explanation for this is that until the very recent acquisition of cloned IFNs it has always been difficult to obtain sufficient amounts of IFN for administration to patients. In fact more successes have been claimed in investigations in which very large doses (10^6–10^7 units) have been given. However it is still not clear how much IFN constitutes a 'physiological dose', since IFNs administered to animals and humans are rapidly withdrawn from the circulation and tissue concentrations are not easy to measure.

On the other hand virologists and clinicians are still loathe to relinquish the ingrained concept of IFN as an antiviral compound. The fact that IFN is commonly produced by the body early in virus infection is no guarantee that the IFN will help to control that infection. In fact it is well known that acute virus infections progress to their peak in the

presence of the induced IFN. The counter argument to this is that once IFN has disseminated and penetrated the key tissues then the acute phase of the infection subsides, while the later humoral and cell-mediated immune responses mount their attack.

Thus the clinical efficacy of interferon is still controversial, although a resolution may be obtained within a few years following current trials with very large doses of cloned material. To date most of the success achieved has been the result of nasal sprays, prophylactic and/or therapeutic, against respiratory virus infections in laboratory animals and in humans. Even low-dose sprays have reputedly been effective in the USSR, Japan and China. In contrast the use of higher dose IFN in scientifically controlled trials in the UK and USA have given more pessimistic conclusions (Greenberg *et al.*, 1978; Scott, 1983). This may reflect undue pressure put on the experimental system, which necessarily uses unnaturally high doses of virus, and in which the IFN is at a distinct disadvantage.

Other viral infections in which IFN has scored some unqualified successes are herpes-simplex keratitis, herpes zoster in immunosuppressed patients, and papilloma infections (warts of various kinds). In the case of keratitis, however, concurrent use of antiherpes chemicals proved more effective. Additional situations, especially those involving chronic infections, are controversial (Scott, 1983; Strander, 1983; Baron *et al.*, 1984).

In many virus infections it is not entirely clear that IFN is working by blocking virus replication directly since IFNs have a multitude of other effects, some of which might be beneficial to the host in combatting the virus, and some of which could indirectly control the spread of virus. For example many viruses, such as papilloma viruses, require actively dividing cells for their replication; thus since large local concentrations of IFN would be expected to decrease the number of dividing cells, these viruses would be inhibited. In respiratory infections the frequent use of nasal IFN spray may relieve the symptoms of infection in the oropharynx and nasal passages, without necessarily decreasing the amount of virus produced, although few sufferers would doubt the value of the treatment.

Another argument which has been used to promote interferon therapy is the fact that it is a natural cellular product, and therefore should be intrinsically safer than chemotherapy. This argument may hold for physiological doses of IFN, but it certainly does not justify the cavalier administration of millions of units in frequent doses. Numerous side-effects have accompanied the use of large systemic doses in patients, although these are usually temporary. Greater caution should in any case be exercised in children and young animals in view of the known ability of excessive IFN to cause runting in young animals (Riviere *et al.*, 1980).

Table 12 represents an attempt to summarize the principal benefits and detriments of IFN therapy. Some of the reservations will undoubtedly be either vindicated or cast aside as a result of clinical trials with cloned IFNs. Hopefully some veterinary trials will also be forthcoming, although the prospect of controlling a respiratory infection within a herd of bovines by repeated nasal spray is something the average farmer would probably not relish. It does not seem feasible to attempt administration of IFN via food or water. In addition the relative species specificity of IFN is a factor against its adoption in veterinary practice, unless there is sufficient overlap between selected species e.g. if bovine IFN turns

TABLE 12. *Features of Interferon*

		Advantage	Disadvantage
(i)	species specific	− (not absolute)	+
(ii)	natural product	+	high doses could be detrimental
(iii)	many types	−	+ possibly different types effective in different infections
(iv)	large doses give side effects	− (usually temporary)	+ especially in young animals
(v)	cloned preparations available	+	−
(vi)	several recognized antiviral mechanisms	+	−
(vii)	may inhibit endogenous IFN production by feedback	+ (insures against too high a dose)	may decrease effectiveness
(viii)	prophylactic and therapeutic	+	−

TABLE 13. *Principal Interferon Inducers*

Natural substances:	Viruses, viral nucleic acids, double stranded RNA, bacteria, rickettsiae, protozoa, endotoxins, mitogens
Synthetic substances:	polyribonucleotides, polycarboxylates, tilorone, propanediamines

out to be effective in sheep and pigs etc. (Babiuk and Rouse, 1977). This remains to be tested with cloned IFNs (Fish *et al.*, 1983).

9.7. INTERFERON-INDUCERS

For many years investigators have attempted to circumvent direct IFN therapy by means of chemical inducers. The early studies were prompted mainly by two difficulties inherent in the direct approach, viz. the limited supplies of IFN available at that time, and the species specificity of IFN. The first problem has clearly been overcome to such an extent that it should now be just as economical to administer large doses of IFN as it would be for inducers. The second problem is still a real barrier and continues to justify the search for more potent and less toxic inducers.

Unfortunately the inducers themselves introduce their own set of problems, such as side-effects, potential toxicity and variable efficacy in different species. In fact not all inducers function in all species (Stringfellow, 1980b; Mayer, 1980). Table 13 lists the chemicals which have been most popular in experimental and clinical situations.

There appears to be no predictable common feature which dictates the efficacy of inducers, with the exception of double stranded polyribonucleotides which convert cells into the 'antiviral state' (see Section 5.5). The lack of a common chemical basis probably stems from the fact that many of these compounds were fortuitously discovered to be IFN-inducers. Many of them may also act indirectly, in view of the observations that some potent inducers *in vivo* are ineffective (or marginally active) in cell-culture systems, e.g. tilorone (Mayer and Krueger, 1980). Some of them may not really be IFN-inducers at all, but may act as immune modulators (see Section 9.8).

Table 14 summarizes the desired properties of inducers. In addition to their general lack of species specificity, another advantage that inducers may have over IFN itself is the prospect of local administration without dissemination in the body. This is just as well since most of them have proven to be toxic when given systemically in animals. In any case one would have to question the deliberate introduction of polynucleotides into an animal. But this does of course restrict their usefulness. The greater success has been reported for poly I:poly C in the treatment of herpes-simplex keratitis in animals and humans, although there have been reports of additional successes against some other viruses in animals (Levy, 1980).

Attempts have been made to improve the therapeutic index of low molecular weight inducers by using chemical derivatives. In the case of tilorone, over 800 derivatives were tested for antiviral activity, but none was more effective than tilorone itself (Mayer and Krueger, 1980). Clearly more research is needed in this area, especially as it pertains to veterinary applications. In this connection it is also worth considering the administration of inducers into feed or drinking water, such as has been accomplished successfully for tilorone in mice (Mayer and Krueger, 1980). This would afford an obvious advantage over IFN therapy.

The result of all these endeavors however may be the ultimate merging of IFN-inducers with immune modulators, such that the distinction between them becomes difficult to

TABLE 14. *Desirable Features of Interferon-inducers*

(i)	non-toxic; minimal side-effects
(ii)	animal can clear or metabolize compound efficiently
(iii)	should be active in many species
(iv)	should be active therapeutically as well as prophylactically
(v)	chemically pure
(vi)	relatively inexpensive
(vii)	single dose (or very few doses) adequate; or incorporation into food and water

draw. But if the consequence of administration of such compounds is the augmentation of host responses to virus infection, then the precise classification of these compounds as IFN inducers or immune enhancers, as well as their detailed mechanisms of action, are of secondary importance.

9.8. Immune Modulators

Immune modulators are substances which either stimulate or suppress one or more elements of the immune system. Immunosuppressive factors induced by viruses have already been discussed in Section 6. The present discussion concerns modulators with potential therapeutic value in virus infections. Few of the known modulators have been exploited to the extent of trials in animals or human subjects (Werner and Zerial, 1984). This has usually been either because of possible toxic effects or because their mechanism of action is completely unknown. This reluctance to try them out is justifiable in view of our realization that indiscriminate tampering with the immune system is a dangerous thing, since an effect on any component of the system will in general be reflected by a counterbalancing trend so that homeostasis is restored. Therefore unless we know exactly what a compound does to the system it is not wise to proceed too far, in case the result is worse than the original disease. Thus more research is needed into the precise effects of modulators on the immune system, and also into the nature of the disturbances caused by virus infections, especially persistent infections. Only then will it be feasible to 'correct' the deficit by appropriate therapy. In the meantime we are stuck with a trial and error approach.

Among the numerous modulators described, two have received widespread attention, viz. levamisole and isoprinosine (Werner and Zerial, 1984). Both have proven effective against a range of virus infections in animals (apart from their antitumor properties), mainly by virtue of their ability to augment cell mediated immune responses, and IFN and NK cell activity. In addition isoprinosine appears to have some direct antiviral effects. The latter has also been effective in shortening the duration of various cutaneous herpes lesions in humans, and possibly decreasing the frequency of recurrences. Unfortunately the exact targets for these compounds are still not known. More detailed knowledge of their actions could lead to their more widespread adoption.

Thus, although the concept of immune modulation as antiviral therapy is attractive, progress is severely limited at present by the lack of knowledge of how these substances act, and how viral-induced dysfunction in the immune system should be corrected.

It has been predicted that it will be possible in the near future to correct any kind of temporary immune dysfunction, whether this is caused by a virus or some other agent, by consuming tablets containing appropriate modulators, which will then restore the immune system to normal. This concept is interesting but is evidently premature. In the meantime it is certainly safer to stick with vitamin therapy, or traditional forms of 'pro-host' therapy.

Chronic immune dysfunction, which invariably results in increased susceptibility to virus infections, might be amenable to correction by an appropriate form of lymphoid cell therapy. In an experimental test of this concept recently it was found that mice could be spared the lethal effects of HSV infection by T-lymphocyte infusions from syngeneic mice (Sethi *et al.*, 1983). The approach might be considered analogous to bone-marrow transplantation for certain cancers, except in the case of viral infections we are probably seeking more specific cell types for therapy. More research is needed to extend this type of approach and to determine its precise effects on the immune system.

10. SPECIFIC ANIMAL VIRUS DISEASES

This section focuses on the major animal viruses responsible for disease, and attempts to review their current and prospective situation, especially in the light of the discussions of the preceding sections.

In a recent brief review, Brown (1981) stated that nobody would argue against the inclusion, in a list of the principal economically important infections, of the six viruses:

foot-and-mouth-disease virus; rinderpest; bluetongue; rabies; Newcastle disease virus; and African swine fever. There would probably be considerable disagreement among Virologists and Veterinarians concerning the next important components of such a list. In this section, bearing in mind the purpose and scope of the review, the Author has also chosen to include herpes viruses, influenza viruses, rotaviruses, selected fish viruses, and bovine leukemia virus. Some of these are better discussed as a group rather than individual viruses. But by no means does this exhaust the list of economically important viruses. Others have been omitted in the interests of brevity, or because they have been less well characterized, or they tend to be more localized (geographically) in terms of disease production. The list will undoubtedly change over the years as some of these viruses are successfully brought under control, while others may assume greater importance.

In order to avoid unnecessary repetition of references, each subsection refers to one or more major sources of information on the particular individual or group of viruses. Additional more specific references may be included within the text. The book by Odend'hal (1983) is a useful source of information on the geographical distribution of many of these viruses.

10.1. FOOT-AND-MOUTH-DISEASE VIRUS (FMDV) (PEREIRA, 1981)

The FMD virus is a highly contagious organism which readily infects cattle, sheep and pigs, occasionally other animals, including humans, but not horses. Epizootics of the disease have been recorded since the 16th century. The disease is worldwide, although it is not endemic in all areas, e.g. North America, Australia and New Zealand, which maintain strict quarantine measures. On the other hand South America records thousands of outbreaks each year.

The principal factors which promote the rapid spread and efficiency of infection are: (i) body tissues and fluids contain very high levels of infectious virus, which may be attained before recognizable clinical symptoms, thanks to a short incubation period; (ii) the ability of the virus to survive (relative to most viruses) in the environment and in meat products; although a pH of less than 5 inactivates the virus; (iii) animals which recover from infection may become chronically infected. This poses a risk of transmission to uninfected animals; (iv) the virus is readily dispersed on prevailing winds and can consequently be conveyed considerable distances over land and water (see Section 2); and (v) the various antigenic types of FMDV do not necessarily confer cross-protection; thus an existing immunity to one strain, by virtue of prior infection or vaccination, is no guarantee of resistance to another strain (Brown, 1981).

The FMDV is a picornavirus, which has been placed in its own genus-aphthovirus (i.e. vesicle producing, a property characteristic of several other types of RNA virus). The virus contains a + strand RNA of 2.5×10^6 daltons. Seven standard serotypes have traditionally been recognized: O, A, C, SAT (Southern African Territories) 1–3, Asia 1. Further subtyping is also possible, although for epidemiological analysis gel electrophoretic profiles of virion polypeptides or RNA digests are now proving more useful (King *et al.*, 1981; Brooksby, 1981). This kind of analysis is essential in many countries because there is a continuous need for vigilance against the emergence of new strains or re-emergence of older strains. An example of this problem has already been presented in Section 4.

The virus usually establishes a respiratory infection, but spreads quickly to other tissues. Vesicles appear in the mouth and on the feet, hence the name of the virus, accompanied by fever. The vesicles then rupture to yield denuded epithelium.

A confirmatory diagnosis of FMDV is readily made in the laboratory by isolating the virus from the vesicular or other body fluids. Distinction from other 'vesicular viruses' is required since the adoption of appropriate control measures is needed quickly.

The control methods differ in endemic and non-endemic areas. Thus in disease-free countries it is important to establish some kind of barrier or 'neutral zone' between such an area and an endemic neighbor. This would involve quarantine regulations and their enforcement for imported animals, and also restriction on the immigration of wild animals

which might be carriers. If an epizootic does arise, slaughter of infected animals and their contacts is certainly the most efficient method of control. The procedure of ring vaccination is often proposed as well to restrict the livestock in the interior in case an epizootic arises from the barrier or neutral zone. It would be desirable to have on hand a suitable chemotherapeutic procedure, in order to save the afflicted animals, or at least their contacts, but as yet no suitable anti-FMDV compound has been produced. Conceivably an interferon-containing spray might be of some value.

In endemic areas vaccination is the only feasible control measure. However, as mentioned above, vaccines are no guarantee of complete protection. The virus itself must mutate frequently, especially considering its rapid rate of proliferation within a herd; consequently novel strains are likely to arise at any time.

The subject of genetically engineered vaccines and synthetic peptides has been given much attention, since this virus has been an attractive candidate for such investigations on economical grounds (see Section 9; Kupper *et al.*, 1981; Kleid *et al.*, 1981; Bittle *et al.*, 1982). They should be preferable to current killed-virus vaccines, but of course they do not alleviate the necessity of being prepared for the emergence of new strains.

Optimistic veterinarians hope that continual vaccination programs, coupled with slaughter of animals infected in localized epizootics, will eventually lead to eradication of the virus from Europe and S. America. But similar prospects for Africa seem dim in view of the abundance of wild carriers in that continent.

One must remember that a virus which has been around for centuries is bound to have negotiated suitable ecological niches in order to ensure persistence.

10.2. Rabies Virus (Murphy, 1977; Koprowski, 1984)

Rabies has long been recognized as a scourge of livestock with occasional and often fatal intrusions into humans and their pets. The virus is essentially worldwide in its distribution, with the exception of the many islands such as Australia, Britain, and Japan. Persistence in the wild is guaranteed by the presence of various carriers, e.g. bats, foxes, skunks, depending upon the location.

Rabies is a rhabdovirus, although it is not serologically related to the well-studied vesicular stomatitis virus (VSV). Nevertheless the two viruses do seem to behave in similar ways in cell culture systems, and this had led to the belief that our extensive biochemical knowledge of VSV can be applied to the rabies virus. For example it has been shown that rabies can persist in mammalian cell cultures, with the accompaniment of defective interfering (DI) particles (see Section 4).

The pathogenesis of rabies has been worked out in detail in rodents. Initially the virus gains access to muscle, sensory organs or skin and there replicates in local unmyelinated (sensory) nerve fibers. Many layers of epithelial cells are susceptible to the virus but the principal target is the unprotected neuron. From here the virus travels by axoplasmic flow, without significant involvement of glial cells or blood cells, to the dorsal root ganglion. It then spreads through the neurons of the ganglion and into the spinal cord, whereupon it ascends rapidly (in a matter of hours) to the brain.

In the brain the major damage is done, resulting in the familiar psychomotor disturbances. In addition the virus spreads into salivary glands (by axoplasmic flow again), from where it is secreted from the acinar mucous cells and transmitted to the victim.

The strict localization of the virus within neurons and salivary glands explains the relative paucity of immune responses, until late in infection when antibody does circulate. It also explains the persistence of the virus in the face of this late response, since salivary glands, as well as the CNS, are relatively inaccessible to immune attack.

Fixed strains of the virus tend to produce more widespread cytopathic effects than the wild or 'street rabies' and consequently stimulate a faster immune response. It has been suggested that the production of DI particles by the wild strain of virus may be responsible for its more limited spread.

Virus infection results in a generalized depression in cell-mediated immunity, although a role for antibody mediated cytotoxic cells (ADCC) has been suggested.

The objective of much research on rabies virus has been the development of better vaccines and therapeutic measures. The presence of large carrier populations in the wild mitigates against eradication of the virus, although strict quarantine measures are for the most part effective in maintaining the present endemic-free areas.

The classical vaccine was a fixed brain-tissue derived preparation which had significant side-effects. A more recent preparation was made from virus-infected diploid fibroblast cells, which were inactivated for use. This was replaced by a subunit vaccine which contained only the viral glycoprotein, and which was theoretically more desirable in view of the ease of mutation of the viral genome (see Section 4) (Norrby, 1983; Koprowski, 1984; Hilleman, 1984).

Considerable effort is now underway however to replace all of these vaccines by immunogenic synthetic peptides or by genetically engineered plasmids and virus vectors (Yelverton *et al.*, 1983; Koprowski, 1984; Kieny *et al.*, 1984). These have all been described in Section 9. It will be interesting to see which of these preparations will eventually prove the most popular. The principal targets for such vaccines would be all the livestock and their handlers who are likely to come into contact with foraging wild animals.

Therapy is a different matter altogether. The present practice of inoculating rabies-immune serum will likely continue for some time.

10.3. African Swine Fever Virus (ASFV) (Wilkinson, 1981; Hess, 1981)

This is a highly contagious iridovirus infection of pigs which is clinically indistinguishable from the totally unrelated hog cholera virus. This illustrates well the concept of different viruses (in this case a DNA virus and an RNA virus) producing similar diseases. Obviously laboratory tests would be required to confirm a diagnosis.

The ASFV is considered the most serious disease of domestic pigs, although it is believed to have existed for a long time in wild pigs in Africa. The disease was only really noticed when domestic European pigs were introduced into Africa.

Since 1957 epidemics have been recorded intermittently in several European countries bordering the Mediterranean, in the Carribean, and in Brazil. Control has been effectively brought about only by slaughter.

The virus replicates in the nucleus of susceptible cells, such as pig leukocyte cultures, in which it also persists. Although pigs appear to be the only species infected naturally, the virus can be adapted to grow in a variety of other cell types and experimentally infects goats and rabbits. In view of this it seems worthwhile considering the possibility of its natural persistence in animals other than swine. Also the significance of its documented persistence in macrophages has been referred to previously (Section 7). The virus also appears to be immunosuppressive (Wardley, 1982, see Section 6).

In this connection it is also important to realize that other iridovirus infections of animals, such as the piscine erythrocytic necrosis viruses, and the frog virus type 3, commonly persist in their natural hosts, usually as innocuous infections (see Section 10). One of the members, lymphocystis virus, produces tumors in a variety of fish species. Therefore it is not surprising that ASFV should persist also in its natural host.

Transmission of ASFV usually occurs through the respiratory route. Following inhalation the virus invades and attacks local lymph nodes and the endothelium of blood vessels, with the result that high titers of virus circulate in the blood and lymph, and eventually various secretory fluids. Different strains of virus are recognized with distinctive degrees of virulence.

An alternative method of transmission is through an insect vector, viz. the *Ornithoderos* genus of tick. This tick occurs in many parts of Africa and in Spain and Portugal. Virus has been isolated from ticks captured in the field. In many of these countries very high titers of ASFV have been found in the gut of the ticks, and there is evidence of transovarial transmission among them. It has been estimated that a single tick contains more than enough virus to infect a pig.

Thus the major routes of virus transmission are believed to be: (i) respiratory, as a result of close contact between pigs; and (ii) tick borne infection. In addition other possible routes

are: (iii) feeding on contaminated meat, in view of the relative stability of the virus and the low threshold of virus required for establishment of infection; and (iv) contact with carrier pigs (other animals?) suffering a reactivated infection.

Laboratory confirmation of diagnosis is traditionally made by virus isolation from blood or visceral tissues, although the development of diagnostic tests based upon a DNA-probe or monoclonal antibodies (see Section 8) would certainly speed up the detection of virus within a herd. There is no doubt, from the evidence of comparative control policies during epidemics in several countries, that prompt diagnosis followed by rapid slaughter of exposed pigs, as performed in France, is economically essential.

Attempts have been made to produce vaccines, but without too much success. Killed virus vaccines have not afforded complete protection against the wild virus, and attenuated viruses, while effective, are not considered consistently safe. There is clearly a desperate need for genetically engineered vaccines. This will however require a considerable amount of research since intensive biochemical analysis of viral genes and proteins have only commenced just recently (Wesley and Pan, 1982). Once the genes responsible for the major antigenic proteins (presumably coat proteins) have been identified, it should be possible to incorporate these genes into bacterial plasmids or vaccinia type vectors. Alternatively it may be possible to prepare a deletion mutant of ASFV, missing several non-essential genes (like the vaccinia prototype vector), which could be used as a vaccine strain. Synthetic immunogenic peptides may also be feasible.

In view of the likelihood of viral-induced enzymes (the virus probably contains more than 50 genes), the possibility of specific chemotherapy, along the lines of antiherpes chemicals, should be profitable.

In the meantime the slaughter policy will probably prevail as the only effective way to prevent the spread of infection. Eradication is at present out of the question; we simply do not know enough about the extent of wild carrier populations.

10.4. Rinderpest Virus (RPV) (Scott, 1981)

Rinderpest virus has been recognized for centuries as the agent responsible for a well defined uncontrollable plague of cattle and buffalo and other susceptible animals. Today it is mainly confined to parts of Africa and Southern Asia, thanks to the availability of the attenuated virus vaccines.

Dissemination of the virus through the ages was brought about by the movement of animals accompanying military campaigns, and in more recent times by livestock transported for commercial purposes. As a result of these ventures the virus was introduced to many parts of the world and into contact with novel host species. Thus humans were directly responsible for ensuring its worldwide distribution, although of course we have no idea how long this virus had been in existence prior to its recognition as an etiological agent of epizootics and pandemics. Conceivably it had already occupied an ecological niche on each continent, including reservoirs or carriers, long before humans intervened and disturbed the balance.

In spite of the obvious success of vaccination programs (when properly applied) in controlling the disease, and as if to remind us of the dangers of complacency, a pandemic erupted in Iran in 1969, apparently from cattle imported from Afghanistan, and spread quickly through the Middle-East. Control measures eventually terminated the pandemic; but nevertheless the potential for further disease outbreaks still exists.

Proposals for eradicating the virus have been made, but in view of the persistence of the virus (and its probable mutation) in wild animals this is not a feasible prospect. It is more important to guard against a repeat of the 1969 pandemic. Rinderpest virus is a member of the paramyxoviridae, and in particular is classified in a genus with measles virus and canine distemper virus, with which it shares significant antigenicity especially in their nucleocapsids. On this basis one might expect some biological similarity between the three viruses. Consequently information gleaned from epidemiological and pathogenetic studies of measles infections should be helpful in understanding RPV infections.

Wild strains of RPV have been adapted to grow in a variety of animals and cell cultures, and it is from the latter that the current vaccines are derived. In view of the widespread demand for rinderpest vaccine, it would seem worthwhile to invest in a genetically engineered vaccine. For example if, as presumed, the major antigenic determinant is one of the virion membrane glycoproteins, then a cDNA copy of the gene coding for this protein could be cloned into a plasmid for growth and expression in yeast or other eukaryotic cells.

The pathogenesis of RPV is manifest in ruminants and swine as an acute contagious disease accompanied by high fever, inflammation, hemorrhage and lesions involving the mucosa of the alimentary tract, probably following initial infection of the nasopharynx. Many lymphoid tissues are infected and appear to constitute the first sites of replication, and as a result leukocyte counts are reduced. Diarrhea results in loss of water and electrolytes and is thought to be responsible for many of the deaths, which may exceed 90% of the early contacts. The virus is eventually found in many body excretions and is probably transmitted by this route to other animals.

Diagnosis can be made on the basis of clinical symptoms and by conventional laboratory tests including cell-culture isolation and detection of antigens and rising antibodies.

The virus is also immunosuppressive (Yamanouchi *et al.*, 1974), probably because of its lympholytic ability (see Section 6), and this state undoubtedly renders the infected animal more susceptible to other secondary infections. In fact this is what we would expect on the basis of our knowledge of other paramyxoviruses, such as measles and NDV.

Prospects for complete eradication of RPV seem dim, except possibly in those endemic areas in which proper cooperation with veterinarians can be acheived. The current vaccines appear to be adequate, provided that continuing vigilance can be assured in order to guard against the possibility of newly emerging strains. Since RPV is a paramyxovirus, it is quite likely that it can persist as a chronic infection in some species of animals.

10.5. NEWCASTLE DISEASE VIRUS (NDV) (LANCASTER, 1981; MOHANTY AND DUTTA, 1981)

This virus is a paramyxovirus, unrelated to rinderpest, which is responsible for outbreaks of Newcastle disease in chickens, turkeys, many other birds, and occasionally humans. The economic impact of the virus is felt by owners of the first two species. The virus is essentially worldwide in distribution, although the disease was apparently not recognized before 1926, when it was described in England and in several Asian countries. The ubiquity of the virus has been enhanced and maintained by a combination of several human and non-human factors, notably: the current husbandry practices in the poultry industry; the incompletely-regulated international trade in avian species; and the migratory habits of many birds. In fact no virus could ask for a better opportunity for establishing a global ecological 'niche' in its host species. Although many birds suffer disease as a result of infection, it is generally agreed that some species, such as parrots, may serve as carriers of the virus.

The relative ease of handling and growing the virus in the laboratory has led to its considerable popularity as a model negative strand RNA-virus for basic biochemical studies in cell cultures. This has also allowed the development of attenuated vaccine strains.

Different strains of the virus exist with markedly differing degrees of virulence, although fortunately these strains are all strongly related antigenically. These differences probably reflect the tropism of the particular strains for specific tissues. Thus some strains are viscerotropic, some are neurotropic, while others may be relatively avirulent. In general the complete clinical picture of a NDV infection represents the contribution of the virulence and tropism factors, together with host determined factors. It will be interesting to determine if the former factors can be ascribed to specific genes of the virus, by analogy with certain other RNA viruses (see Section 4). The outcome is a disease with variable clinical manifestations, which can range from death to asymptomatic infection. Transmission is easily acquired by the aerial route on droplets. Diagnosis is made by conventional laboratory tests.

Birds which survive the infection are immune to the virus, but little consideration has been given to the likelihood of virus persistence. In the case of the poultry industry this factor is probably not important since the birds do not persist long themselves. In wild birds however, stress, brought on by a long strenuous flight, or sudden climatic changes, could reactivate a latent or chronic infection. Likewise a domesticated bird might reactivate the virus in response to stressful transportation. In either case the reactivated and excreted virus would be available for transmission to susceptible hosts. In view of these considerations it is worthwhile reiterating the fact that during persistent infections mutants will arise and will be selected for or against depending upon the local environment. Thus 'novel' highly virulent strains could conceivably arise at any time. This may even explain the apparent sudden 'emergence' of NDV in 1926. Relatively avirulent strains may have predominated for some time prior to that year, until the 'balance' was altered in an individual carrier or a population of birds.

The prospects for eradication of NDV, or even restricting its spread, seem to be minimal. It is clearly more practical at present to ensure a continuous supply of adequate vaccines. In addition it would seem worthwhile continuing the search for therapeutic chemicals for emergency use.

10.6. Bluetongue Virus (BTV) (Sellers, 1981)

This virus affects mainly sheep, occasionally some deer, but has no effect on cattle and goats. There are other related orbiviruses (e.g. Ibaraki virus) which have somewhat different spectra of sensitivity. The disease is not contagious, but is transmitted by midges. Although fetal death often accompanies infection in pregnant ewes, the major problem in adults is a loss in general condition and decreased milk production.

The disease was first described in the 19th century in Africa, from where it spread to the Middle-East, USA and more recently to Europe.

Bluetongue virus, and its 20 or so relatives, constitute the members of the BTV complex, alluded to already in Section 4 (a genus within the reovirus family). They are typical orbiviruses containing 10 double strand segments of RNA. In view of the genome structure one would anticipate considerable opportunity for gene recombination or reassortment between individual types. In addition to replication in the natural mammalian hosts, the orbiviruses, unlike other reoviruses, also replicate within insect vectors. This feature may affect the prospect of genetic recombination, and may also provide a different set of selection pressures on recombinant and mutant viruses which arise.

The BTV is normally transmitted by *Culicoides* midges, although other vectors have also been implicated. The geographical distribution of the midge determines the limits of spread of the virus, and explains why the disease has defined northern and southern borders. Outside this global belt of disease the temperature is too low for the vector. Within the belt the midges can travel many kilometers on prevailing winds, thus assuring efficient spread of the virus. The lifecycles of the midges vary in different areas; this must have some effect on the seasonal aspect of the disease.

The pathogenesis of infection is determined by the feeding habits of the midge, which allow ready access of BTV to blood and lymphoid tissues. The virus replicates in the endothelial cells of the blood vessels, and this produces the characteristic edema and hemorrhaging. Titers of up to 10^7 infectious virions per ml of blood can be attained, which is evidently enough to infect other midges. In addition the virus crosses the placenta, so that fetuses are infected, with fatal consequences. Virus is also found in semen. Diagnosis is not always easy, since the clinical syndrome is not unique, but confirmation can be obtained by viral isolation in cell cultures.

It has been suggested that the virus persists in blood cells. The concept of BTV persistence was supported by recent studies in cell cultures, in which the virus was able to establish a long-term persistent (possibly latent) infection (Hallum *et al.*, 1984). This is important since it suggests the possibility of a reservoir of BTV in the wild, perhaps in some species of wild sheep, deer or bovines (Luedke *et al.*, 1977).

Control of the disease by midge spraying has been attempted, but the insects evidently recolonize the sprayed zone from other areas. Frequent dipping of sheep helps to reduce the number of feeding insects.

Live attenuated virus vaccines are available, but they are considered risky in view of the possibility of reversion to virulence in the vector, and the inevitable risk to a fetus in pregnant ewes.

Once again here is a disease with clear need for an engineered vaccine or synthetic immunogenic peptide. Presumably one of the 10 segments of RNA contains the gene for the dominant antigen, and a cDNA copy of this could be incorporated into a bacterial plasmid (for protein production) or a vaccinia-like vaccine.

But even if this is accomplished, the potential for the emergence of novel strains of BTV and its relatives remains. No suitable antiviral chemical has been described, although it would be useful to have one for emergency use.

10.7. HERPES VIRUSES (MOHANTY AND DUTTA, 1981; WITTMANN *et al.*, 1984)

Herpes viruses are ubiquitous, and many species of animals have several distinct apparently unrelated herpes viruses. It appears that each animal may also have its own cytomegalovirus (CMV) in addition to other herpes viruses. In terms of their biological and pathogenetic properties the herpes viruses fall naturally into several subfamily groupings, although detailed classification at present is premature. Nevertheless the CMVs clearly constitute a group of their own with internal consistency, and likewise some of the so-called lymphotropic herpes viruses (i.e. associated primarily with specific classes of lymphocytes) show internal consistency. The others however show considerable diversions in molecular and biological properties.

But these considerations can be misleading, for even within the CMV group there is apparently no nucleotide sequence homology between the viruses of different species (Huang and Pagano, 1974), and even the genome structure differs basically, although the size of their genomes is approximately the same, about 240,000 nucleotide pairs (Mercer *et al.*, 1983; Ebeling *et al.*, 1983). This is significantly larger than other herpes virus genomes, which range from about 120,000 to 170,000 nucleotide pairs (Hayward *et al.*, 1984). The lack of correlation between genome organization and biological properties is particularly well illustrated by recent studies on rodent CMVs. Thus, the rat CMV has internal repeated sequences in its DNA very similar to HCMV, and hence can exist in four orientations (Meijer *et al.*, 1984). In contrast the guinea pig CMV, which is especially suitable as an animal model for HCMV because of the similarity in biological attributes, has a genome organization more like MCMV, i.e. without internal repeated sequences (Gao and Isom, 1984).

It is not at all clear whether the members of the CMV group arose from a single primordial CMV which adapted itself to numerous species, followed by frequent mutations but with preservation of biological attributes, or whether they represent independent herpes viruses which inevitably attained analogous ecological niches, manifest as common tissue tropisms and behavioral patterns. The apparent lack of nucleotide homology between them at present argues against the first alternative. A few herpes viruses however, do show significant homology, e.g. HSV-1 with HSV-2; the equine herpes viruses types 1 and 3; and Mareks disease virus of chickens with the herpes virus of turkeys (HVT) (Hayward *et al.*, 1984). This suggests common ancestry in these cases, although related sequences could represent common host sequences, since several herpes viruses have been found to contain one or more regions of sequence homology with mammalian DNA (Peden *et al.*, 1982; Puga *et al.*, 1982; Ruger *et al.*, 1984).

Most of the herpes viruses were discovered accidentally as a result of inadvertently reactivating them from their characteristic persistent infections in their natural hosts. This includes those of veterinary concern. It should be noted too that a consequence of increasing stressors, due to intensive husbandry and farming practices, is an increase in overt herpes diseases.

It is beyond the scope of the present article to describe these viruses in detail, but it is worth highlighting the economically important members, bearing in mind that additional members will probably surface in the near future.

At least five bovine herpes viruses have been described in association with disease (Ludwig, 1984). The one best studied is bovine herpes virus type 1 or infectious bovine rhinotracheitis virus (IBRV), which can cause respiratory, genital, conjunctival or encephalitic infections, as well as abortions. The virus becomes latent in the trigeminal ganglion, much like HSV, from which it can be reactivated. Other sites of latency are also likely to exist.

Other bovine members are bovine mammilitis virus (bovine herpes virus 2), bovine herpes virus 3 (malignant catarrhal fever virus), bovine herpes virus 4 and bovine CMV. These are all associated with different diseases in a variety of domestic and wild bovines. Vaccines are available for some of them, but one has to question the usefulness of this concept for the control of viruses which are ubiquitous and probably persist throughout the bovine world.

Three equine herpes viruses have been described, viz. type 1 (equine rhinopneumonitis virus; equine abortion virus), type 2 (which does not appear to have a descriptive epithet), and type 3 (equine coital exanthema virus).

Types 1 and 3 have been implicated in persistent as well as acute clinical disease (Burrows and Goodridge, 1984).

Pseudorabies virus, PRV (Aujesky's disease virus) is the most notable porcine herpes virus, which has a well documented history of epizootics, especially in Europe. The virus can establish infections in many different mammalian species, including bovines. The virus principally affects the CNS, respiratory and reproductive tissues. In the former case the symptoms bear some resemblance to those of rabies, hence the name, pseudorabies. The other attribute, the so-called 'mad-itch', refers to the nervous irritation in localized infected areas of the skin.

The use of PRV vaccines has been alluded to (see Section 4). Both live and killed virus preparations are available, and these are commonly used in some European countries. The advantage of the live vaccines is the fact that they have specific deletions in the DNA which decreases their virulence (Lomniczi *et al.*, 1984a,b). Accordingly the chances of reversion to virulence are low (Section 4). Pigs also have their own CMV, which is thought to be quite prevalent in porcine species.

Among the many avian herpes viruses which have come to our attention, the most important ones in economic terms are Marek's disease virus (MDV) and infectious laryngotracheitis virus (ILTV), both of which cause serious problems in chickens. MDV only establishes a productive infection in feather follicle cells, from which substantial titers of virus may be shed into the environment and hence to nearby chickens. In addition the virus is oncogenic as a result of its ability to infect (though not replicate) and presumably transform lymphoid cells. These then infiltrate nervous tissue and produces the familiar paralysis associated with classical Marek's disease. Various host and viral factors, however, such as age and breed of chicken, and strain of virus, affect the outcome, with the result that the clinical picture is variable (Ross *et al.*, 1981; Payne, 1982). The immunosuppressive aspects of MDV have been described already (Section 6).

Vaccination against MDV is possible by means of live turkey herpes virus (HVT), which unfortunately has to be administered to each individual bird, or by live attenuated strains of MDV. Both types of vaccine apparently protect the birds from lymphoma formation by wild MDV, although the latter is still replicated and shed. Therefore uninfected contacts will still receive virus.

Several fish herpes viruses have now been characterized (Wolf, 1982, 1984). The first one recognized was channel catfish virus (CCV) which has an economic impact on the catfish farming operations in Southern USA. This virus was isolated originally from epizootics in catfish. It is normally associated with covert persistent infections of this species, and is only reactivated by stressors such as increased water temperature.

Salmonid herpes viruses have been isolated, again from epizootics, in rainbow trout in

the USA and in trout and masu salmon in Japan. The former has been characterized to some extent, and is unusual in that its replication is optimal at 10°C, which of course befits its role as a pathogen of cold water fish (Wolf *et al.*, 1978). Other herpes viruses have been seen in Turbot in Scotland (Buchanan and Madeley, 1978), Walleye in Canada, and Pacific cod (Wolf, 1982). All of these viruses are strictly species specific and apparently unrelated. No doubt numerous other herpes viruses exist in aquatic lifeforms, awaiting their turn to be discovered by Fisheries Virologists.

10.8. INFLUENZA VIRUSES (MOHANTY AND DUTTA, 1981; SCHILD, 1984)

These viruses are subdivided into types A, B and C, which resemble each other biochemically and biologically, except for the apparent restriction of B and C viruses to the human population. Numerous strains of type A are found worldwide throughout mammals and birds. Some strains are endemic within a given population, while other strains may be carried within that same population. This is particularly evident for birds, which in addition to possessing their own disease-producing strains, can carry and transmit other strains (see Section 2).

Each type A virus possesses eight segments of single stranded RNA, corresponding to eight genes and 11 proteins. The three largest genes code for the P1, P2, P3 proteins, which together comprise the transcriptase complex. The NP gene codes for NP protein, which is associated with the virion genome; the HA gene codes for the two subunits of the hemagglutinin (HA) protein; the NA gene codes for the neuraminidase. Different reading frames are utilized by the other two genes: the M gene which codes for the prevalent matrix (M) or M1 protein and the minor M2 protein; and the NS gene which gives rise to two non-structural proteins NS1 and NS2. The envelope proteins hemagglutinin (HA) and neuraminidase (NA) constitute the major immunogenic and virulence determinants. Accordingly epidemics and pandemics have been correlated with changes in the HA or NA proteins, or both, such that the population does not have complete immunity against the new virus strains. Presumably epizootics can arise in analogous fashion.

The influenza A viruses are best envisaged as a global pool of genes assorted into 13 HA subtypes (H1–H13) and nine NA subtypes (N1–N9). From such a pool any permutation is theoretically possible and in fact many have been documented, especially amongst avian isolates. Drifting among other virion proteins evidently occurs, as expected, but this is thought to be of little significance for viral disease and epidemiology.

The human strains exist as three HA types and two NA types. Pandemics were associated with the generation of the H1N1 strain in 1918; the H2N2 in 1957; the H3N2 strain in 1968; and to a smaller degree the re-emergence of H1N1 in 1978 among the non-immune population. These are the so-called antigenic shifts referred to in Section 4 and which resulted from genetic reassortment or recombination among HA and NA genes. Between these years antigenic drift in HA and NA proteins resulted in epidemics. It is thought that shift is restricted to type A viruses because of the opportunity for recombination with animal strains. B and C strains only appear to undergo drifting, hence only epidemics result. However, in consideration of the 'genetic flexibility' of influenza viruses and their unpredictability, it remains to be seen how long this dogma survives.

All HA and NA types have been documented among the avian viruses. Thus numerous recombinants are possible. Diseases range from mild symptoms to mortality. A variety of tissues in addition to the respiratory tract may be affected. Probably the most notorious example is the fowl plague virus (FPV) (H7N7) which has killed innumerable chickens and turkeys during its 100-year documented history. Because of the widespread distribution of the avian viruses and their hosts, and their migratory habits, it has been difficult to keep track of the epidemiology of these viruses. For this reason when an epizootic does arise in, for example, chicken flocks, it is not possible to say for certain whether the culprit arose from recombination with an enzootic strain, or whether it was due to a completely exotic virus making its first contact with the non-immune flock. In contrast human records are much more detailed and consequently the epidemiology of the human strains is much better understood.

The epizootic among harbor seals in 1979–80 (Section 4) was apparently due to a strain of H7N7, which may thus have been acquired from birds (Geraci *et al.*, 1982).

Swine influenza is an important respiratory infection of domestic pigs in North America, Europe, USSR and other countries. Mortality is rare, but the infection can be transmitted to human handlers (Ottis *et al.*, 1982). There is clearly crossover with human strains since the swine virus has been designated HswN1 to indicate that the NA protein is very similar to the human NA of N1 strains, although it is officially regarded as a variant form of H1N1. In fact there has been discussion concerning the species of origin of human and swine influence A viruses, and it has been suggested that the one was derived from the other. However, it is also possible that both may have arisen independently by recombination with other preexisting virus strains.

Two strains of equine influenza have been described in association with epizootic respiratory infections in horses (H3N8 and N7N7). The viruses have a worldwide distribution. The infection spreads rapidly among horses of all ages, but mortality rate is low. Some drifting in the major antigens has been observed. Vaccination with preparations of both killed virus strains is commonly done; consequently immunity against the existing strains is continuing to spread. This may eventually force selection pressures on mutant strains as they inevitably arise, with the result that novel recombinants may appear in the future. Nevertheless, horses tend to have a higher value placed upon them in comparison to other livestock, and therefore protection against a debilitating respiratory infection is important, especially since influenza virus is immunosuppressive (Section 6) and potentially capable of making the animal susceptible to secondary infection.

Thus, in view of the worldwide distribution of influenza viruses among many species of mammals and birds, and their potential for recombination and persistence, there does not appear to be much prospect for their eradication or control. The virus appears to have established an enviable niche to ensure its prolonged survival. Continuous vaccination of important species is therefore justified, but constant vigilance is required for the emergence of new recombinant strains, which will demand new vaccines. The recent avian epizootic referred to in Section 4 was a reminder of this. It would also seem to be worthwhile reconsidering the use of antiviral chemicals for emergencies. In this connection adamantine has been used successfully in controlling the spread of influenza in humans and in experimental animals (Oxford and Galbraith, 1984).

10.9. Rotaviruses (Mohanty and Dutta, 1981; Flewett and Babiuk, 1984)

It has now become evident that rotaviruses constitute the major causes of acute diarrhea in young animals and humans. They are for the most part species specific, and representative viruses have been isolated from many species of livestock, wild mammals and birds. In neonatal livestock such as calves the disease can be fatal. The viruses appear to be globally distributed, although most records of the disease come from the more temperate climates, where the disease tends to be seasonal, being most frequent during the winter months. It is quite likely that many adults serve as carriers, in which the virus persists.

As members of the reovirus family, the rotaviruses share the common properties of a double-shelled capsid, and the genome is segmented double-stranded RNA although, in contrast to the 10 segments characteristic of reoviruses and orbiviruses, the rotaviruses have 11 segments. Thus, the opportunity for genetic recombination or reassortment between different strains is high, and there does appear to be a pool of diverse strains within a given species of the virus. Gel electrophoresis of the RNA segments readily detects strain differences by virtue of alterations in the mobility of specific segments. In addition some diseases have been associated with two coexisting strains of the virus.

Persistent infections have been described in cell culture models (Misra and Babiuk, 1980), therefore it is likely that the viruses can persist in nature, although their possible association with chronic gastro-intestinal diseases has apparently not received much attention.

Transmission is via the fecal–oral route, and this is facilitated by the very high titers of infectious virus found in feces of diseased animals. In fact the easiest method of diagnosing the agent is to examine negatively strained fecal specimens under the electron microscope. If the specimen is positive, then within minutes the characteristic clusters of rotavirus particles are clearly visible (McLean and Wong, 1984).

Pathogenesis is initiated in the small intestine, in which the virus infects and kills the epithelial cells of the villi and consequently interferes with adsorption. Watery feces, which may be contaminated with blood and mucus, are produced, and the eventual loss of water and electrolytes may lead to dehydration and death.

Infections are accompanied by antibody responses, especially gut IgA, and it is the presence of antibodies in colostrum which protects calves from infection at birth. When maternal antibodies decay however the calves become very susceptible to the virus. The age dependency may conceivably be due in part to the stage of maturation of the G.I. tract.

Diagnosis is best made by electron microscopy, as mentioned above, or ELISA type assays for detecting viral antigens. Cell culture isolation is more difficult to achieve.

An attenuated bovine rotavirus vaccine is available, although it would have to be administered immediately after delivery to be effective. Otherwise the most effective control measures are good sanitary practices and the immediate isolation of diarrheic animals.

10.10. Fish Rhabdoviruses (Pilcher and Fryer, 1980a; Wolf, 1976, 1982)

Among six documented rhabdoviruses of fish, two have been regularly associated with epizootics of salmonid populations, viz. the viral hemorrhagic septicemia virus (VHS, Egtved) in Europe and elsewhere, and the infectious hematopoietic necrosis virus (IHNV) in parts of North America. The other apparently less notorious members are the carp viruses: spring viremia of carp (SVC virus) and swim bladder inflammation (SBI virus); pike fry rhabdovirus (PFRV); and the more recently isolated Rio Grand Perch (cichlid) rhabdovirus (Wolf, 1982). All of these viruses were isolated from fish which had suffered reactivated infections as a consequence of stress. The initial five viruses were compared with respect to their biophysical and biochemical attributes by Hill *et al.* (1975).

The impact of IHNV on North American salmon stocks has been illustrated in Section 1 (Leong and Barila, 1983). Probably equally impressive data could be obtained for VHS in Europe, and other countries. The following details refer specifically to IHNV, but an analogous story could be constructed for VHS and the other rhabdoviruses.

The IHNV apparently originated on the west coast of North America, from which it spread across the continent (including Alaska) and recently to Japan, probably as a result of inadvertant transportation of infected carrier fish stocks or eggs.

Young salmonids of several species are particularly susceptible to IHNV, which replicates to quite high titers in the kidney. Since the anterior kidney is the major lymphopoietic and erythropoietic tissue, then afflicted fish become anemic. They eventually die from electrolyte imbalance, but not before they have excreted substantial amounts of virus into the environment, from which other fish can be infected. Probably IHNV infected fish would be highly susceptible to secondary microbial infection as a result of immunosuppression.

Mature healthy fish are generally resistant to IHNV, although they, as well as surviving young fish, may become persistently infected. It is now well known that reactivation of the virus in apparently healthy fish can be stimulated by various stressors (Hetrick *et al.*, 1979b; Wedemeyer *et al.*, 1984). An example of this is spawning, in which case the eggs may become infected (Traxler, 1983; and personal communication). In this case the situation may be aggravated by the fact that the migrating salmon also have to adapt from salt water to fresh water, which itself must be a potent stressor.

The water temperature is also an important factor in determining susceptibility of salmonids to IHNV-disease, and conversely fish which are already infected are much more sensitive to a temperature rise. For example in one experimental study, IHNV-infected rainbow trout died rapidly following an increase in water temperature of 2°C, whereas uninfected fish survived (Hetrick *et al.*, 1979a).

In general small changes in the quality of the water, including the presence of certain metals, reduced O_2 tension and other parameters, which by themselves may not be lethal, can nevertheless alter the balance in a persistently infected fish in favor of the virus (Hetrick *et al.*, 1979b).

Cell culture models persistently infected with IHNV have been described which apparently resemble those of the more familiar mammalian rhabdovirus VSV (Engelking and Leong, 1981). Since short DI particles have also been described for IHNV, then it is logical to presume that the mechanisms involved in persistent IHNV infection are similar to VSV.

The non-piscine rhabdoviruses have shown a tremendous adaptability to different hosts. Therefore at first sight the apparent narrow species specificity of the piscine members is surprising. However other factors may be involved in limiting their spread in the aquatic environment, in view of the experimental work which showed that the fish viruses could replicate in a variety of mammalian and reptilian cell cultures maintained at poikilothermic temperatures (i.e. characteristic of cold-blooded animals) (Clark and Soriano, 1974). Thus these viruses do have the potential for successfully spreading to other hosts. It may be that, in spite of the absence of visible fish kills, the fish rhadboviruses are quite capable of establishing limited or covert infections in other species of fish.

A study is in progress to compare the different strains of IHNV from different regions of the west coast of USA. Evidently different strains exist, and they can be typed by serological analysis or by comparison of their virion polypeptide profiles by gel electrophoresis (Leong *et al.*, 1981). Major differences reflect changes in the envelope glycoprotein and the nucleocapsid phospho-protein. As a result of applying this technique to isolates from 25 different regions it was evident that a given strain of virus tended to become endemic in a region, regardless of the fish species present. In other words it is the environmental selection pressure, not the host species, which determines which virus mutant will establish an ecological niche in a given area. Presumably this prevalent strain could be supplanted at any time by a better adapted mutant, although this process might require considerable time.

It has been assumed so far that transmission of IHNV, or other rhabdovirus, is accomplished simply by shedding virus into the water, whereupon it is taken up through the gills of other fish. The virus does appear to be relatively stable in water (for an enveloped virus), but contact would have to be made within a day or two of its excretion in order to ensure infection of a recipient. Eggs can also serve as vehicles of transmission, either in the form of internally infected ova or as virus adventitiously bound to the exterior.

The possibility of vectors of transmission is worthwhile considering. Birds have been shown capable of transporting infectious IPNV (Section 2) without the necessity of its replication, in their guts followed by fecal excretion. Since birds of various kinds, as well as mammals, feed upon spawned fish carcasses, it seems likely that IHNV could be transmitted by this route. The virus is probably more stable in fish tissues and fluids than it is in water.

Parasites of various kinds are abundant in water habitats; consequently the opportunity for transmission by this route would seem to be as profitable (for this virus) as it is for terrestrial vectors. But to date only one example has been examined, namely the calicivirus San Miguel sea lion virus (see Section 2).

Vaccination of fish against rhabdoviruses is a difficult prospect to envisage, even though it is probably only necessary for fry or juveniles. For practical reasons it would have to be restricted to hatcheries, although even then it would be labor intensive to inoculate individual fish. To some degree these difficulties have been overcome by circumventing the need to handle individuals. The most practical alternative is the use of a bath vaccination, in which groups of fish are immersed in a vaccine containing vessel, from which the animals absorb the killed or live attenuated virus (or protein components) through the skin and gills. Such a technique appears to be successful for bacterial vaccines (Egidius and Anderson, 1979). The use of spray vaccination, in the manner proposed for chicken vaccination in broilers, should be worth considering. The major obstacle to any kind of

vaccine administered via the water route is the inevitable dilution effect. Genetically engineered vaccines or synthetic peptides, made by methods analogous to rabies virus products, are feasible, but the problem of administration is still a formidable barrier.

For this reason chemotherapy is an attractive alternative, although the chosen chemical would have to be completely non-toxic to other aquatic wildlife. This is true whether the chemical is applied to a large body of water or a contained hatchery situation, since effluents from a hatchery eventually mix with rivers or lakes. No suitable chemical has yet been proposed.

Thus, for the immediate future the prospect of controlling rhabdovirus infections will likely be better if stressors are avoided, and the current policy of killing diseased fish and their contacts is maintained. This latter measure could probably be decreased to some extent if fish populations were routinely monitored for the presence of IHNV, and if carriers were physically separated from virus-free fish. Diagnosis for IHNV is done easily in a laboratory which carries common fish cell lines. The presence of carriers could be ascertained by deliberately stressing randomly selected animals, in order to reactivate the virus.

10.11. OTHER FISH VIRUSES (HILL, 1981; WOLF, 1982)

The most intensely studied fish virus is infectious pancreatic necrosis virus (IPNV), which is a birnavirus containing two segments of double stranded RNA. It exists in several serologically distinct strains with a very broad host range and has been recorded in most of the trout farming areas of N. America, Europe and Japan. Apart from marine and freshwater teleost fish, many species of shellfish are also susceptible and in fact several strains were isolated from them.

The virus readily infects most known fish cell lines *in vitro*, with rapid and extensive cytolytic effects. Thus laboratory diagnosis is straightforward. Persistence of the virus in carrier fish is probably common, since fish surviving an infection often shed the virus for prolonged times, (see Section 7.3.3) and it is known that the virus can be reactivated by stressing apparently healthy fish. It is possible that IPNV may characteristically persist in non-salmonid species such as suckers and in shellfish.

Lymphocystis virus is an iridovirus found in many species of fish in fresh water and marine habitats. The virus replicates very slowly, *in vitro* and *in vivo*. Replication is accompanied in connective tissues of the host by a bizzare hypertrophy of the infected cells, so that microscopic tumor-like growths, the lymphocysts, appear on the surface (Lopez *et al.*, 1969). They are unsightly (making them non-marketable) but are not fatal. Nevertheless the possible detrimental effects of large numbers of these cysts has not been investigated. In fact very little biochemistry has been done on this intriguing system, although the recent successful cloning of the viral genome into plasmids should pave the way for fruitful experiments (Darai *et al.*, 1983).

An interesting group of viruses associated with epizootics of several wild species has recently come to light. These are the erythrocytic necrosis viruses (piscine erythrocytic necrosis virus—PEN virus), which have been independently isolated from the east and west coasts of North America and have been given various epithets (Appy *et al.*, 1976; Walker and Sherburne, 1977; Evelyn and Traxler, 1978; MacMillan and Mulcahy, 1979). Different viruses differ somewhat in their precise sizes and morphology but they all appear to be iridoviruses, and they are invariably found in the immature erythrocytes of dead or moribund fish, where they form into discrete crystalline arrays or inclusions visible by light microscopy. Similar viruses have been identified in cod and blenny off the English coast (Smail and Egglestone, 1980).

The east coast isolates were obtained from various species of fish, but on the west coast they have been specifically associated with epizootics in pacific salmon and in *Clupea* (herring). Survivors of these epizootics have shown dramatically reduced hematocrits, an observation which suggests that the virus is the etiological agent of the anemia. In fact it has been suggested that this erythrocytic necrosis virus (VEN) might be the cause of the cyclic fluctuations in populations of marine salmonids and herring (Evelyn and Traxler,

1978). In support of this idea is the known difficulty in obtaining the overtly diseased fish in most years. This point, together with the lack of success in propagating the virus *in vitro*, has hindered progress in its study.

The incidence of recorded virus infections among wild, farmed and aquarium fishes is continually increasing. To some extent this reflects better and wiser observations, and the availability of numerous cultured cell lines. But it also probably reflects increased stressors (especially pollutants and other results of human intrusions), which are becoming more prevalent in natural waters; as well as increased handling; transportation; forced adaptation to new environments; and unnatural intermingling of species. It is already clear that we can expect the same gamut of viruses, from the same families, as their mammalian and avian counterparts. But in reality the total number of individual piscine viruses, even for just the teleosts, is likely to exceed those of mammals etc. because of the considerably greater number of teleost species (i.e. about 22,000 teleosts compared with 4400 mammalian species). In view of this realization, prospects for vaccination are bleak. Instead it seems more reasonable to think of controlling the viruses, at least on small scales, by judicious chemotherapy. In the wild, fish have managed to contain their infections over the years without help from us, and will hopefully continue to do so if they are permitted to continue breeding.

10.12. Bovine Leukemia Virus (Straub, 1981; Kaaden and Lange, 1984)

All vertebrate species probably possess retroviruses, and it is likely that all these viruses are oncogenetic in the appropriate conditions. Often the economically important animals do not survive long enough to realize this oncogenetic potential, but in cattle, especially dairy herds, this can be a problem. It has now been established that the so-called enzootic bovine leukosis is due to bovine leukemia virus (BLV), which is biochemically similar to the well-studied avian and murine leukemia/sarcoma viruses, although there is no antigenic or genetic relationship with them.

The presence of the virus has been documented in most parts of the American and European continents, as well as in other scattered areas.

The genome of BLV is a single stranded RNA, approximately 10,000 nucleotides long, containing the terminal repeat sequences (LTR) characteristic of the family, and the virion also contains the usual assortment of bits and pieces of cellular RNA.

Normal bovine DNA is evidently devoid of BLV-like sequences, as shown by nucleic acid hybridization tests between tissue DNA and either purified viral genomic RNA or cloned DNA derived from the viral genome. This indicates that the virus is exogenous to the host and has created optimism in terms of programs of eradication in European countries. Analogous hybridization tests confirmed the lack of cross-reaction with other retroviruses.

Disease is usually manifest in older animals, and initially presents as a lymphocytosis due to elevated numbers of B-lymphocytes, which may be the result of viral transformation. This condition may be symptomless, but it can progress to lymphosarcoma in a variety of tissues, whereupon visible tumors may be evident. The latter condition is usually associated with poor health and performance. Cellular DNA in lymphocytes and tumor tissues from diseased animals usually contains multiple copies of integrated (proviral) BLV-DNA.

The details of the integration and transformation processes are still being worked out. It appears that on the average more integrated copies of BLV-DNA are found in lymphocytes from animals with persistent lymphocytosis than in tumour cells. Also there is uncertainty as to the specificity or otherwise of the integration sites (Kettmann *et al.*, 1983). Thus, the overall process of oncogenesis in BLV-infected cattle may be somewhat different from the other retroviruses studied so far.

Persistant viremia leads to easily detectable circulating antibody, which thus confirms the presence of the virus within a herd. The virus itself or viral antigens can also be detected by laboratory tests.

The virus can also produce disease in sheep and buffaloes. In addition viral replication has been demonstrated in pigs, *in vivo*, and in cultures derived from human, simian, equine, ovine, caprine and bat cells *in vitro*. The oncogenic potential of the virus and the wide spectrum of cellular susceptibility to it have raised concern about the health hazard to handlers. However no human sera have been found positive for BLV antibodies, and accordingly the risk of transmission to humans is presumed negligible. In fact the problem of how transmission occurs between cattle is still a puzzle, in view of the difficulty in demonstrating virus in the secretions and excretions of diseased animals. Horizontal transmission is presumed to occur normally by contact, and this would serve to explain the epidemiology of the viral disease, but it is not known how. A possible role of vectors has been suggested on the basis of experimental transmission by biting flies and ticks, but the importance of such a route is difficult to ascertain.

Virus has been found in colostrum and semen. Thus it is possible that a variety of routes is available. Concern has also been raised about the consequences of virus in semen and possibly in ova, in view of the worldwide transport of bovine sperm and ova for breeding purposes (Eaglesome *et al.*, 1980; Kahrs *et al.*, 1980). Clearly it would be desirable to ship only materials certified to be free of BLV-genes.

The whole issue of virus persistence, in the symptomless bovine and carrier animals, is one that needs to be addressed, but at present not enough is known about the biology of the virus. Meanwhile, some European countries, notably Denmark and West Germany, are proceeding with slaughter and confinement policies in efforts to eradicate the virus, together with the administration of killed virus vaccine preparations, which are partially protective against the disease.

11. CONCLUSIONS AND PROSPECTS

The success of animal viruses in evolution has been assured by four general attributes; genetic variation; various methods of transmission; efficient replication within host cells; and the ability to persist in the host. As a consequence of these properties, animal viruses have adapted to every species of animal on earth, and have occupied numerous 'ecological niches', with the result that humans and their livestock suffer widespread, barely-controllable diseases.

Genetic variation occurs mainly by mutation, which has a high temporal frequency by virtue of the relatively rapid rate of replication of viral genomes in general, and is particularly high for RNA viruses, which evidently do not possess 'proofreading' or correctional capabilities. Consequently, RNA viruses comprise pools of genetically-related genomes rather than discrete nucleotide sequences. These are subject to intrinsic and extrinsic selection pressures. In addition viruses containing segmented RNA genomes have the capacity for 'recombination' (gene reassortment) between genomes, and in practice this event occurs quite frequently and can lead to pandemics.

Animal viruses have exploited all the opportunities for transmission within a herd or species, and between species. They have commonly made use of vectors, notably arthropods. But it appears that numerous other animal species can also serve as vectors and can engage in complex infection cycles. The presence of so many vectors and intermediaries helps to confound our attempts to control the spread of viruses.

Animal viruses have evolved a number of strategies of replication. For this reason it is unlikely that a general antiviral approach will work. Instead it is more likely that relatively virus-specific approaches for control will be more practical.

Animal viruses commonly persist within the host. This is an attribute which has received less than its due attention over the years, and in fact it appears that persistence is a normal consequence of virus infection. Probably every animal contains hundreds of persistent viral genomes. Persistence may be manifest as a true latent infection, in which viral gene expression is absent or restricted; or it may be chronic, in which case the virus may replicate to some degree and may be accompanied by cell and tissue pathology. Chronic infections may be held in check by one or more components of the immune system, and they continue

to give rise to mutant forms of the genome. Any kind of persistent virus infection could conceivably interfere with essential or non-essential cellular functions.

The state of the persistent infection is undoubtedly influenced by the many factors to which the cell is subjected, as well as other host factors. Consequently an apparently quiescent virus infection may at any time be reactivated and lead to a disease episode.

Among the factors which are known to cause reactivations are those commonly defined as stressors. Livestock and fishes are being subjected to increasing numbers of stressors, in the form of such things as pollutants, increases in population density, changes in husbandry practices, etc. with the result that the balance between host and persistent virus is frequently tipped in favor of the virus.

An important corollary to these considerations is the realization that the reactivated virus may not be genetically identical to the original infecting virus; its biological properties may thus be different. Possible consequences of such mutations are: changes in tissue tropisms and virulence; resistance to antibodies or cytotoxic lymphocytes; resistance to antiviral chemicals; acquisition of immunosuppressive ability; and others.

The prospects for control of animal viruses, at least for several specific viruses of veterinary concern, are quite good; prospects for eradication are dismal.

Vaccines are entering a new era, thanks to the advent of genetic engineering and peptide-synthesis techniques. Thus cloned, plasmid-derived virion proteins, and their chemically synthesized analogs, will undoubtedly compete with existing veterinary vaccines. In addition the concept of a virus (such as vaccinia) as a vector for other viral genes is an attractive one which has gained instant popularity, notwithstanding several possible pitfalls. This is an exciting development which can be exploited beyond the realm covered by the experimental results to date. Unfortunately vaccines will always be daunted by the fact that viruses frequently change and consequently the host may not be fully protected against a new form of virus.

For this reason alone it is essential to continue the search for useful antiviral chemicals. Furthermore there will probably always be a need for therapy in addition to prophylaxis. The relative success achieved recently against herpes viruses has raised optimism about the future of antiviral chemicals. There are limitations however, and one must always be prepared for disappointing clinical trials, due to undesirable side-effects of the chemical, the emergence of resistant viruses, and other problems.

Other forms of therapy are now available, based upon different considerations of the virus and the host, and no doubt novel approaches will arise in the near future. Conceivably the correction of a viral-induced homeostatic imbalance in the host, e.g. by administration of immune modulators or other biological response modifiers, may turn out to be a simpler, more effective way of controlling viruses in the future; but at present we do not know enough about the interactions between host and viral factors to justify the use of this approach.

In any case viruses are here to stay, and they will continue to evolve. We can only hope to keep track of their changes by constant monitoring, so that we can devise ways of restoring the precarious balance in favor of the host.

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