PRINCIPLES AND PRACTICE OF CLINICAL VIROLOGY

EDITED BY

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Preface

There has been a spectacular increase during the last thirty years in our knowledge of virology. This has taken place to such an extent that virology can now be regarded as an umbrella term encompassing a variety of distinct but related disciplines. There are fundamental connections with biochemistry, genetics and molecular biology, and each of these aspects would be worth a treatise in itself. Clinical virology is that aspect which is concerned with the cause, diagnosis, treatment and prevention of virus infections of man. It too has acquired a substantial body of knowledge and accumulated experience over the past thirty years and this book is intended to be an authoritative account of the present situation. Formerly virological diagnosis was time consuming, retrospective and rarely influenced the management of the patient. During the past 10–15 years the picture has changed dramatically. Newly recognized diseases such as AIDS and some haemorrhagic fevers which have very serious consequences for individuals and populations have been shown to be due to viruses. In the clinical virology laboratory there has been a change in emphasis towards rapid diagnostic techniques. Finally, effective anti-viral chemotherapy is a reality at least for some virus infections and there has been an expansion in the use of immunoprophylaxis. Thus the current principles and practice of clinical virology are concerned with rapid laboratory diagnosis leading to appropriate patient management which might involve specific therapy and/or infection control measures at a hospital, a national and occasionally at an international level.

In organizing the book we were aware that there is no single arrangement that is entirely satisfactory. We have chosen to arrange the chapters on the basis of individual viruses or groups of viruses. General chapters on virus structure, taxonomy and pathogenesis are not included but the information on these aspects necessary for an understanding of the practice of clinical virology is included in the individual chapters.

Clinical virology is a subject which continues to evolve. This is usually for one of two reasons, either the need to apply new technology or the need to study new diseases or epidemiological situations. We have therefore invited authors who are specialist investigators into each of the viruses to contribute up-to-date, stimulating accounts of the practice of clinical virology and provide a framework for the assimilation of imminent advances. One chapter has already had to be significantly updated during the time of preparation of the book and it is our intention, with new editions, to remain up-to-date as the subject advances.

A J ZUCKERMAN
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Chapter 1.i

Herpes Simplex

M. Longson

THE HERPESVIRIDAE

The full extent of the family *Herpesviridae* can only be guessed, but it is certainly vast. At least one representative member has been found in nearly all animals where a search has been made; new species are continuously being added. At present there are probably more than 100 different known herpesviruses, and in a comprehensive catalogue Roizman (1982) lists over 90 officially recognized family members. The host range spans the whole spectrum of warm- and cold-blooded vertebrates and invertebrates, from green sea turtles to green monkeys, from mice to humans (Table 1.i.1). The viruses share common structural features and many chemical, antigenic and biological properties. They are all double-stranded DNA enveloped viruses with icosahedral capsids (100 nm diameter) made up from 162 hollow capsomeres. The envelope of all viruses in the family contains lipids, hence organic solvents destroy virus infectivity and intact envelopes are impermeable to negative stains.

As expected because these are DNA viruses, maturation of progeny begins in the nucleus where viral nucleic acid is incorporated into the core of newly synthesized capsids. These

TABLE 1.i.1 REPRESENTATIVE* LIST TO ILLUSTRATE BREADTH OF HERPESVIRIDAE FAMILY

Animal host	Virus
Cat	Cat cytomegalovirus
Catfish	Channel catfish virus
Cattle	Allerton virus
Cattle	Infectious bovine rhinotracheitis virus
Cormorant	Cormorant virus
Dog	Canine herpesvirus
Domestic fowl	Marek's disease virus
Duck	Duck plague virus
Elephant	Elephant herpesvirus
Falcon	Falcon inclusion body disease virus
Gnu	Malignant catarrh of wildebeest virus
Guinea-pig	Guinea-pig cytomegalovirus
Horse	Equine abortion virus
Human	Epstein–Barr virus
Human	Herpes simplex virus
Monkey	African green monkey cytomegalovirus
Monkey	B virus
Monkey	Patas monkey herpesvirus
Mouse	Mouse cytomegalovirus
Pig	Aujesky's virus
Pigeon	Pigeon herpesvirus
Rabbit	Herpes III virus of rabbits
Salmon	Herpesvirus of salmon
Snake	Cobra virus
Toad	Lucké virus
Turtle	Green sea turtle virus

*Only illustrative; selected randomly and for no other reason.

then bud through the inner lamella of the nuclear membrane to accumulate in the space deep to the outer lamella. The budding process provides part of (if not all) the viral envelope; infectivity is thereby acquired and the virion is transported through the endoplasmic reticulum to the cell surface. Typical and highly pathognomonic intranuclear inclusions are formed in cells which have borne active virus replication (see Figures 1.i.9 and 1.i.10). Because many herpesviruses can fuse the cells they infect, polykaryocytes readily appear in affected tissues. Thus, for well over 50 years, both medical and veterinary practice have accepted inclusions (Lipschütz bodies) and polykaryocytes (Tzanck cells) as hallmarks of herpetic pathology.

The nomenclature and hierarchical classification of herpesviruses are contentious and no satisfactory or universally acceptable system has yet emerged. In the eyes of many virologists, historical names are enshrined in tradition even if they are trivial (vernacular) and are disallowed by the International Committee for the Taxonomy of Viruses. In the case of the herpesviruses of man there is, in the vernacular nomenclature, a common language universal to everybody (physician, nurse, microbiologist, technologist and dedicated worker), a quality hardly enjoyed by the current 'official' system. In Table 1.i.2 the medically important agents are described and given both trivial and official names.

Man is the natural host to four herpesviruses and, in addition, can be a very rare victim to an accidental infection with one of the simian agents – B virus. Various biological properties, a number of antigenic features and the molecular profile of their constitutent nucleic acids readily separate these four viruses from each other and from all other *Herpesviridae*. In contrast, they are distinguished neither by electron microscopy nor by the morphology of the inclusion bodies they provoke. Although there are major differences between the four viruses, they share many attributes, such as absolute identity of capsid morphology, near identity in replicative cycles and a universal propensity to establish a life-long latent infection. To a lesser extent these four viruses manifest many common epidemiological features and there is common ground in many aspects of pathogenesis. An understanding of the natural history of any one of the four human herpesviruses leads to a general understanding of the other three. Between the four viruses there are a number of common chemotherapeutic targets, but the antigenic differences which separate them preclude any immunological cross-protection.

Herpes simplex virus (HSV), varicella-zoster virus, human cytomegalovirus and Epstein–Barr virus are all agents of major concern in medicine. Each is considered separately in this volume and the following sections are limited to a consideration of HSV.

TABLE 1.i.2 OFFICIAL AND COMMON NAMES FOR HUMAN HERPESVIRUSES

Official nomenclature*		Trivial name
Family	*Herpesviridae*	
Subfamily	Alphaherpesvirinae	
Species	Human herpesvirus 1	Herpes simplex virus 1
	Human herpesvirus 2	Herpes simplex virus 2
	Human herpesvirus 3	Varicella-zoster virus
Subfamily	Betaherpesvirinae	
Species	Human herpesvirus 5	Cytomegalovirus
Subfamily	Gammaherpesvirinae	
Species	Human herpesvirus 4	Epstein–Barr virus

*Adopted by the International Committee for the Taxonomy of Viruses.

INTRODUCTION

Herpes simplex is a clinical description for a vesicular exanthem, classically sited at the vermillion border of the lips, where skin and mucous membrane meet. Identified as probably infectious by Vidal in the middle of the nineteenth century, in 1913 the cause of the eruption was established and the new virus was given its name.

The word 'herpes' itself has a fascinating history which dates back into antiquity (Beswick, 1962). Born in Sanscrit tradition, translated into hippocratic medicine, adopted by Anglo-Saxon scholars, enshrined in dermatological nomenclature (Morton, 1694), the word was commonly used as the radical in many clinical descriptions of disease but has now become the almost exclusive property of virology.

THE VIRUS

General Properties

Herpes simplex virus (HSV) is officially a member of the subfamily Alphaherpesvirinae and exists as two serotypes, HSV type 1 (HSV-1) and HSV type 2 (HSV-2). The antigenic differences which separate the two are relatively minor, and HSV-1 and HSV-2 are perhaps best thought of as intratypic variants of a single virus species and not as true serotypes in the full meaning of the term (see 'Strain differences'). On the other hand, the differences between HSV-1 and HSV-2 extend beyond the purely serological, and these differences will be emphasized as is necessary in the sections which follow. As an alphaherpesvirus, HSV is a rapidly growing, cytolytic virus which establishes itself in a 'latent' form in nerve tissue. In these ways it is distinguished from betaherpesviruses (slow growing, cytomegalic, mesothelial latency) and from gammaherpesviruses (lymphoblastoid cell associated).

Man is HSV's only natural host (gibbons, marmosets, owl monkeys and shrews can catch the virus if brought into contact with man, but are not infected in the wild). Experimentally, the virus can be made to infect many non-primates, yet this is an artificial situation and does not detract from the essentially privileged association between HSV and man.

Architecture and Chemical Composition

By electron microscopy the virus is virtually indistinguishable from other herpesviruses. There are four basic structures, an envelope (overall diameter 110–220 nm), a tegument, a nucleocapsid (diameter 95–105 nm) and a DNA containing core (Figures 1.i.1 and 1.i.2). The envelope appears to be trilamellar and almost identical in architecture to cell membranes. However, there are on the surface of the envelope, structures or embedded 'spikes' about 8–10 nm long and 5 nm apart. These are viral glycoproteins synthesized by infected cells and incorporated in their membranes, prior to virus release by budding through those membranes. Often the envelope is seriously distorted, thus giving the virion a grotesque shape. The tegument is an ill-defined fibrillous area between the envelope and the nucleocapsid.

The nucleocapsid is icosahedral and is formed from 162 capsomeres, each long, hollow and polygonal. Most are hexagons measuring just under 10 nm in diameter, with a prominent axial hole about 4 nm in diameter. In electron microscope preparations, it is common to find both naked (i.e. unenveloped and non-infective) and enveloped virus particles, as well as many virions containing two (or more) nucleocapsids within a single envelope. The core (75 nm diameter) consists of an electron-dense toroid with a less dense central mass. Occasionally, coiled structures can be observed and these undoubtedly represent the DNA of the virus. In many preparations there are empty particles ('doughnuts') in which the central region of the capsid is penetrated by negative stain. In some cases such appearances are artefactual, but in others they represent coreless virions – which are non-infective. An area, sometimes called the pericore, lies between the core and

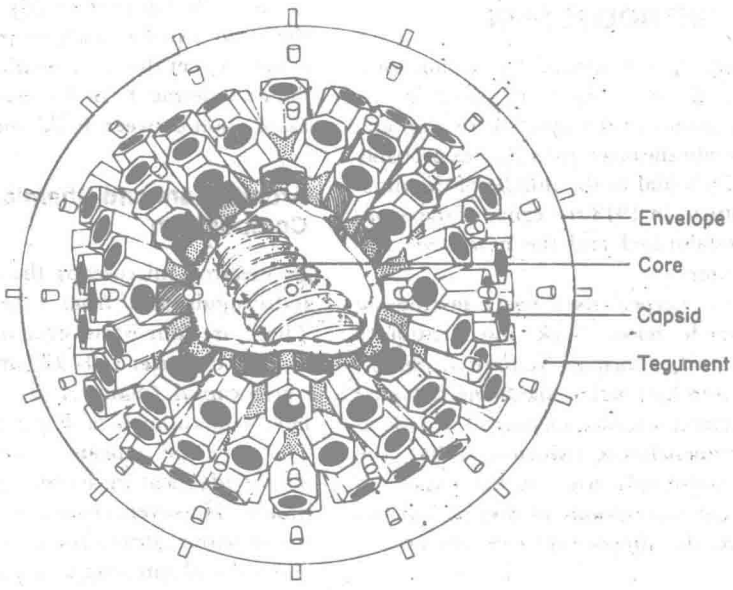

Figure 1.i.1. Idealized illustration of herpesvirus virion, showing envelope with surface projections, tegument, icosahedral capsid with 162 hollow polygonal capsomeres and DNA core.

the capsid and may contain additional structures.

The buoyant density of the virion in caesium chloride measures 1.251–1.281 g/cm^3, according to the strain and the source of the virus; HSV-1 is very slightly lighter than HSV-2.

Chemical composition

Suspensions of HSV are notoriously difficult to purify; no method is entirely satisfactory. Inevitably, suspensions are contaminated with host material which can corrupt all manner of immunological, chemical and molecular data. Techniques used to remove contaminants are often inadequate and frequently damage the very material they are supposed to purify.

In the broadest terms, HSV contains 60–80 per cent protein, 20–25 per cent phospholipids, 6–7 per cent deoxyribonucleic acid and 1.5 per cent sugars, but accurate measurements are well nigh impossible.

Herpes simplex virus lipids are limited to the viral envelope and are exclusively of host cell origin. There are at least 40–50 structural polypeptides in HSV, although many of these may be precursors of others and the whole area of HSV protein analysis is bedevilled by intractable problems of purification. Most of these proteins are associated with the nucleocapsid and are either polypeptides or phosphoproteins, but up to eight are glycosylated and belong to the envelope. These envelope glycoproteins are identified as gA, gB, gC, gD, gE and gF, and some may well have a determining role in the pathogenesis of herpetic disease. In addition to the virus envelope, they can be detected in infected host cells. Generally, very little is known about their function. They play a role in virus infectivity, in virus attachment to and in virus penetration into host cells. The fusion of HSV-infected cells is related to gA and gB (which are probably two different forms of the same protein), whereas gC is absent from strains of HSV which spontaneously produce multinucleated giant cells. Glycoprotein D on the membrane of infected cells is in some way related to cell-mediated cytotoxicity; on the

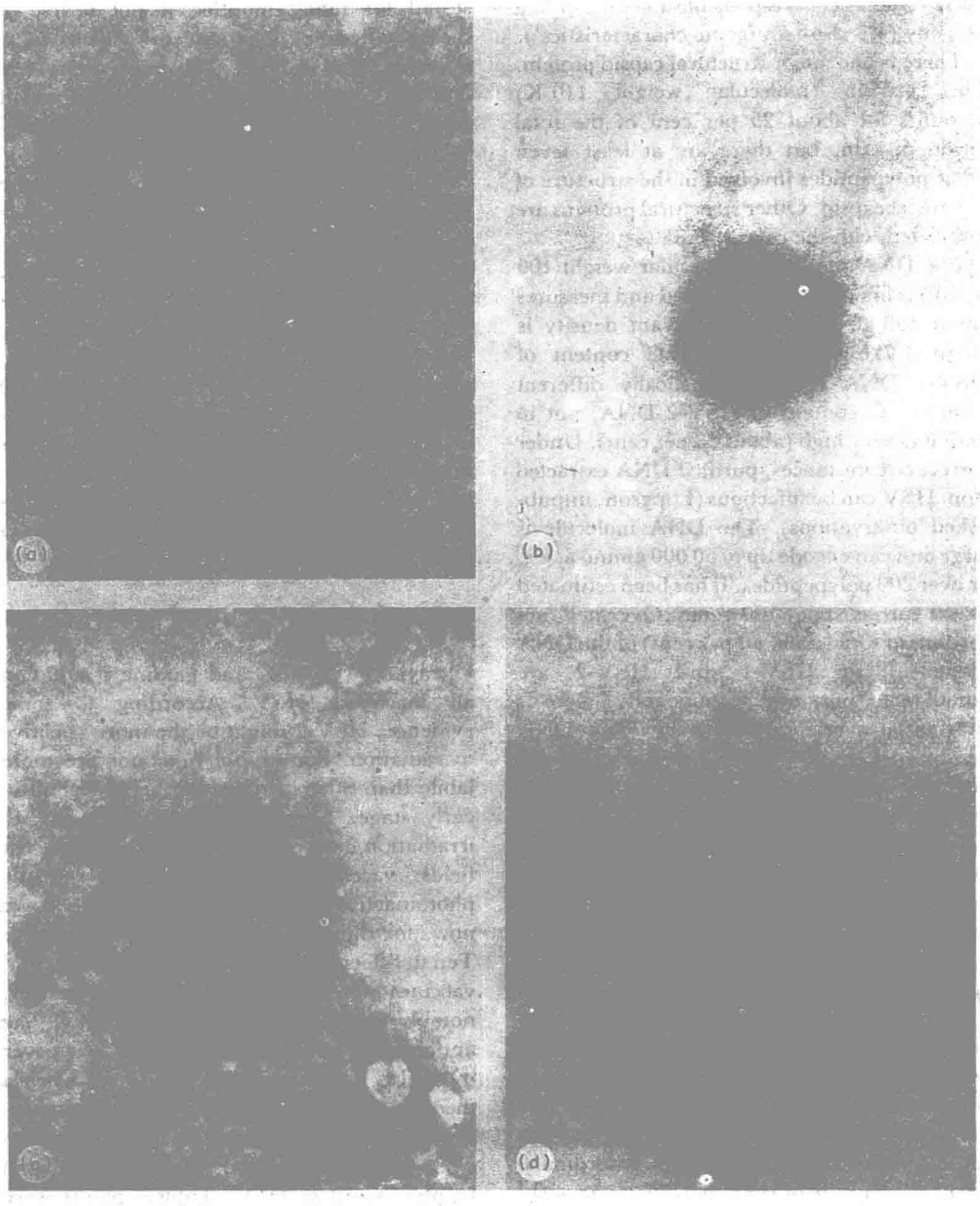

Figure 1.i.2. Electron microphotographs of HSV-1 particles, showing: (a) complete virion with envelope; (b) naked empty particle ('doughnut'); (c) naked particle with core; (d) naked particle revealing hollow capsomeres. (Courtesy of Dr D.J. Wood.)

other hand, gE is responsible for Fc binding capacity (see also 'Antigenic characteristics').

There is one major structural capsid protein. This protein (molecular weight 110 K) accounts for about 25 per cent of the total virion protein, but there are at least seven other polypeptides involved in the structure of the nucleocapsid. Other structural proteins are associated with the core of HSV.

The DNA molecule (molecular weight 100 $\times 10^6$) is linear, double stranded and measures about 150 kb pairs. The buoyant density is about 1.726 g/cm^3. The G + C content of HSV-1 DNA is characteristically different from G + C content of HSV-2 DNA, but in both it is very high (about 68 per cent). Under correct circumstances, purified DNA extracted from HSV can be infectious (Longson, unpublished observations). The DNA molecule is large and can encode up to 50 000 amino acids, or over 200 polypeptides. It has been estimated that it carries about 100 genes. Over half (according to some data, 70 per cent) of the DNA sequences in HSV-1 and HSV-2 are homologous but, most significantly, there is substantial overlapping between HSV DNA and human DNA. On the other hand, there is virtually no genetic homology between serologically unrelated herpesviruses. A detailed knowledge of the genetic anatomy and chemistry of HSV is essential to a proper understanding of herpes virology. For a review and references, see Nahmias, Dowdle and Schinazi (1981).

Resistance to heat and disinfectants

Herpes simplex virus is fragile, with a relatively poor resistance to heat and extracellular environments. It is thermolabile, although this property is influenced by ion and protein content of the surrounding fluid. In ordinary media the half-life of HSV at 37°C is variously quoted as between 90 minutes and 14 hours, but the constitution of the fluid is all-important. In distilled water, as opposed to balanced salt solutions, the virus tends to be more stable. Cell-free suspensions of infective virus can be stored for many months, if not years, in bicarbonate-free medium to which 50% skimmed milk – or 50% glycerol, or 5–10% fetal calf serum, or 35% sorbitol – is added prior to refrigeration at 4°C or – 70°C (stability at – 20°C is unreliable). Alternatively, HSV can be successfully freeze-dried, or it can be preserved indefinitely with dimethylsulphoxide in liquid nitrogen.

Ether in water (20%) and other lipid solvents, such as 70% alcohol (not absolute) or chloroform, completely inactivate HSV. Similarly, most common disinfectants (5% phenol, formaldehyde, glutaraldehyde, 1:10 000 quaternary ammoniums and 0.3 p.p.m. hypochlorites) rapidly abolish infectivity. Other virucidal compounds include detergents, chlorhexidine, merthiolate, sodium azide, β-propriolactone and some proteolytic enzymes such as papain.

Effect of radiation

Ultraviolet (UV), X and gamma irradiation all inactivate HSV. According to some evidence, HSV-2 might be the more sensitive to radiation. Some viral functions are more labile than others, infectivity being lost at an early stage. The sensitivity of the virus to irradiation has been exploited in two different fields: vaccine production and therapeutic photoinactivation. Both applications have now, for different reasons, been discredited. Ten to fifteen years ago, UV-inactivated HSV vaccines were licensed in various countries, notably Bulgaria, France and Germany. The antigenic potency of these vaccines was never in doubt, but many were withdrawn when a theoretical oncogenic potential was recognized.

Phototherapy relied on the ability of certain dyes (proflavine, neutral red, methylene blue) to photosensitize HSV. Topical paints were used on skin lesions prior to exposure to visible light. Initial enthusiasm for this form of therapy was short lived; controlled trials soon revealed its lack of efficacy, and doubts were raised about safety.