REVIEW ARTICLE

Unity and Diversity in the Herpesviruses

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INTRODUCTION

In this review we consider some attributes which may be characteristic of herpesviruses and some properties which distinguish between them. As we shall show, the systematic interpretation of information we now have on a few herpesviruses presents us with some difficulties. For many members of the group we are spared this embarrassment only by the virtual absence of information beyond the initial recognition of the existence of the virus and the allocation of an arbitrary label. Thus, although more than 70 viruses may be listed as presumptive members of the group, criteria allowing their inclusion vary from a brief description of particle morphology (e.g. viruses observed in snake venom, oysters and fungi) to the extensive antigenic, structural, biochemical and genetic characterization of the virus [e.g. herpes simplex virus types 1 and 2 (HSV-1, HSV-2)]. Moreover, the number of separately identifiable viruses which are currently attributed to particular hosts probably reflects the degree of interest in the host species and its afflictions rather than any preferential distribution of viruses. Consequently, independent isolates of viruses from one host which have been given a single name or label may be more heterogeneous than a set of individually named viruses from the same or another host. In our references to a particular virus, or 'species' of herpesvirus, we will therefore use the trivial name by which it is most widely known. Whilst these names have been arbitrarily and, at times, unfortunately chosen, alternative proposals (Roizman et al. 1973) have not been consistently applied and no better general suggestions have yet been made. For our present purpose we also prefer to retain the non-systematic term herpesvirus group to denote those viruses which conform to part or all of the following description: 'Viruses of eukaryotes with linear, double-stranded DNA genomes of more than \(8 \times 10^6\) mol. wt. which are replicated in the nucleus of infected cells, assembled into 100 nm diam. icosahedral capsids composed of 162 prismatic capsomeres which are enclosed in glycoprotein and lipid (ether sensitive) envelopes to give the normally infectious extracellular form of the virus.'

In the sections which follow we shall be mainly concerned with a consideration of common and diverse properties of herpesvirus genomes and gene products as measures of the unity and diversity of the group. In doing so we neglect, for the present, reported differences in growth, ultrastructure and morphogenesis (see, e.g., Watson, 1973). However, it would indeed be a myopic account of the properties of a group of viruses which failed to consider their hosts and the resulting disease. What can usefully be said here, however, may be said briefly, as follows:

(i) Herpesviruses have been observed in hosts as diverse as a fungus, oyster, fish (e.g. channel catfish, rainbow trout), amphibians and reptiles (e.g. Lucké virus of frog renal adenocarcinoma), birds (including Marek's disease virus of chickens) and numerous mammals including monkeys, apes and man (Nahmias, 1972).

(ii) A single host may be inhabited by multiple distinct herpesviruses with differing
consequential diseases (e.g. man is the natural host for at least five viruses – HSV-1, HSV-2, varicella-zoster virus, Epstein–Barr virus and human cytomegalovirus).

(iii) A property of many herpesviruses is that they persist in their natural hosts for prolonged periods after the primary infection as latent or asymptomatic infections and give rise to periodic recurrences of mild disease. The normal mode of horizontal transmission is direct transfer of virus from body fluids of asymptomatic animals to intimate contacts.

(iv) Herpesviruses of vertebrates may be crudely divided into three major groups based on the cell types involved in infection and persistence in the natural host and on the effects on related hosts (Barahona, Melendez & Melnick, 1974). Group one is formed of viruses which cause little or no overt disease in the natural adult host but which can give generalized infection of neonates or immunologically compromised adults. Most of the viruses in this group are designated cytomegaloviruses and include agents from man, monkeys, pigs, horses and many other mammals. The viruses are normally species-specific in vivo and in cell culture (Wright, 1973). Viruses of group two may be generally designated 'lymphotropic' herpesviruses and characteristically cause a mild, transient lymphoproliferative disease in their natural host and persist in lymphocytes. However they cause severe, frequently fatal lymphomas and leukaemias in related host species or in the natural host in the presence of accessory genetic or other factors. We may divide this group into Group 2(a), comprising ‘B-cell tropic’ viruses such as Epstein–Barr virus in man, herpesvirus papio and chimpanzee leukocyte virus(es), and Group 2(b) comprising ‘T-cell tropic’ viruses such as herpesvirus saimiri and atelos and Marek’s disease virus (Deinhardt, Falk & Wolfe, 1974; Frank, Andiman & Miller, 1976). The third group consists of viruses which cause self-limiting primary skin or respiratory tract infections of varying severity in the natural host. These viruses typically persist as latent infections of the central nervous system and give periodic recurrences of mild disease but may cause severe infections involving the central nervous system in a different host animal. Typical examples are herpes simplex, B-virus, herpesvirus tamarinus, SA8 and varicella-zoster together with similar viruses of monkeys.

Whilst host and disease represent important expressions of unity and diversity these divisions are artificial since they result from subjective evaluation of complex properties. By contrast the information in the following sections is capable of at least some quantification.

Common and idiosyncratic features of herpesvirus DNAs

Size, gross structural features and kinetic complexity

Estimates of the mol. wt. of HSV DNA range from 82 to $100 \times 10^6$ (Roizman & Kieff, 1975) and studies of at least seven other herpesviruses have given DNA mol. wt. in the same range. The significance of some differences in the values reported for other herpesviruses is not always clear but it seems certain that the DNA of channel catfish virus is smaller than that of most other herpesviruses (P. Sheldrick, N. Berthelot & S. Chousterman, personal communication) and that murine cytomegalovirus DNA is significantly larger (Mosmann & Hudson, 1973). At the moment a DNA mol. wt. of $>80 \times 10^6$ may provisionally be considered a group characteristic but possible systematic differences in size or kinetic complexity remain to be evaluated. Measurements of the kinetic complexity of HSV-1 DNA (Frenkel & Roizman, 1971) are consistent with the majority of the genome being composed of unique sequences and therefore capable of encoding information for more than 100 proteins of 40000 mol. wt. If size differences are confirmed and are not due to the contribution of reiterated or redundant sequences they would represent considerable differences of genetic capacity which we should see reflected in the range of proteins we are presently capable of detecting.
Kieff, Bachenheimer & Roizman (1971) noted that the majority of single-stranded molecules from alkali-denatured DNA of HSV-1 or HSV-2 were less than half the mol. wt. of the intact duplex. Similar observations have been reported for DNAs from Marek's disease virus (Lee et al. 1971), Epstein–Barr virus (Nonoyama & Pagano, 1972; Pritchett, Hayward & Kieff, 1975), murine cytomegalovirus (Mosmann & Hudson, 1973), pseudorabies virus (Ben-Porat et al. 1976) and, interestingly, channel catfish virus (P. Sheldrick, personal communication). Whilst the biological function of these single-strand interruptions remains to be determined, it may be that this property of the genome is a characteristic of the group.

An interesting sub-group characteristic of herpesvirus DNAs is suggested by recent studies which have shown differences in the arrangement of unique and reiterated or 'palindromic' nucleotide sequences in various representatives of the group. Sheldrick & Berthelot (1974) reported the seminal observation that the HSV-1 genome contained contiguous internal inverted repeats of terminal sequences and had terminal redundancies revealed by exonuclease digestion. These workers suggested that such a sequence arrangement might give rise to mutual inversions of ‘long’ and ‘short’ regions of unique sequences bounded by these palindromes and thus to a population of molecules containing four isomeric arrangements, differing in the relative orientation of these two sets of unique sequences. These predictions have been vindicated and a precise clearly stated model developed and confirmed (Hayward et al. 1975; Wadsworth, Jacob & Roizman, 1975; Clements, Cortini & Wilkie, 1976; Skare & Summers, 1977) and the DNA of HSV-1 may now be represented in simplified form as illustrated in Fig. 1. Thus ‘long’ (L, \(70 \times 10^6\)) and ‘short’ (S, \(9.4 \times 10^6\)) unique
Fig. 2. For legend see facing page.
sequences are combined in each of the four possible orientations by virtue of their being enclosed by non-identical palindromic \((abLb'a'\) and \(acSc'a'\)) sequences which share the small cryptic terminal redundancy \(a\). The DNA of HSV-2 has a similar overall arrangement of redundant and unique sequences and likewise gives rise to four isomers differing in the relative orientation of \(L\) and \(S\) unique sequences and experiments with bovine mammilitis virus are also consistent with the existence of four isomers (T. Buchman & B. Roizman, personal communication, Class 3, Fig. 1).

Although excellent progress has been made on the description of this genome structure (Wilkie, 1976; Jones, Hayward & Roizman, 1977; Skare & Summers, 1977) its genesis and functional implications remain unclear and it may well be premature to erect an elaborate theoretical edifice upon it. Nevertheless, studies on a limited number of other herpesviruses have established that this sequence arrangement is not universal and it would be disconcerting if such differences were purely molecular-biological serendipity. A simplified summary of present information on the gross arrangement of unique and redundant base sequences of these other viruses is also shown in Fig. 1. It is tempting to discriminate three strategies based on the number of orientations of unique DNA sequences which are present in the respective populations of mature DNAs. Thus, pseudorabies virus has a single set of palindromic sequences which surround a ‘short’ unique sequence and which apparently mediate inversion of this short unique sequence relative to one orientation of the ‘long’ unique portion of the genome, giving rise to two isomeric forms (D. Powell, J. B. Clements &

\[\text{Fig. 2. Estimates of mean nucleotide composition [mean } \% (G + C), \text{ indicates human viruses] and gross measures of intramolecular heterogeneity of nucleotide distribution for herpesvirus nucleic acids. Solid bars indicate estimates for the 5 to 95 \% limits of the } \% (G + C) \text{ distributions derived from the corresponding points of the thermal denaturation transitions. Other measures of intramolecular heterogeneity of base composition based on the appearance of sub-fractions with distinctive buoyant density after shearing of high mol. wt. DNA (\(\bullet\) and broken lines indicate mean composition of sub-component derived from a molecule with an initial mean nucleotide composition indicated by square symbols), or on multicomponent thermal denaturation transitions [\(\triangle\) indicates midpoint of sub-component curves translated to } \% (G + C) \text{ scales]. Abbreviations of virus names and sources of information additional to that presented by Ludwig (1972a); Wildy (1973); Goodheart & Plummer (1975) and Roizman & Furlong (1974) are: B – B virus (H. Ludwig, personal communication); Pr – pseudorabies (Graham et al. 1972); SpM – spider monkey herpesvirus, cebid herpesvirus 3; IBR – infectious bovine rhinotracheitis (Graham et al. 1972); HSV-2 herpes simplex virus type 2 (Kieff et al. 1972); SqM – squirrel monkey herpesvirus, herpesvirus tamarinus; HSV-1 – herpes simplex virus type 1; ECE – equine coital exanthema, equid herpesvirus 3; Tree shrew – tree shrew herpesvirus, Tupaiid herpesvirus 1; BMV – bovine mamillitis virus; EB-H/B – Epstein-Barr virus, HR-1 and B95-8 (Pritchett et al. 1975); MuCMV – murine cytomegalovirus (Mossman & Hudson, 1973); HuCMV – human cytomegalovirus; GCMV – guinea pig cytomegalovirus; ECMV – equine cytomegalovirus, equid herpesvirus 2; EAV – equine abortion virus, equid herpesvirus 1; Catfish – channel catfish herpesvirus; Frog-4 – frog virus 4 (Gravel, 1971); MaCMV – marmoset cytomegalovirus; SA6 – simian cytomegalovirus; RhCMV – rhesus cytomegalovirus; VeCMV – vervet cytomegalovirus; Trout – herpesvirus salmonis, herpesvirus from rainbow trout (Wolf et al. 1976); Rat – cytomegalovirus from Rattus; FeILT – feline infectious laryngotracheitis virus; VZ – varicella-zoster virus; H. saimiri – herpesvirus saimiri [infectious middle (M) genomes of 90 x 10^6 are composed of about 70 \% of light (L) non-reiterated sequences with a mean \(\% (G + C)\) of 36 \% and terminally located ‘heavy’ sequences totalling 30 \% of the molecular length but composed of multiple copies of a sequence of about 2.8 x 10^6 mol. wt. with a mean density of 71 \% (G + C), Bornkamm et al. 1976]; Rabbit – cottontail rabbit herpesvirus, herpesvirus sylvilagus [value of 66 \%(G + C) cited for ‘lapine’ herpesvirus (Bachenheimer et al. 1972) presumably refers to the virus isolated from New Zealand White rabbits by Nesburn (1969)]; Dog – canine herpesvirus [a value of 66 \%(G + C) was given by Aurelian (1969) but repeated measurements on the same and other isolates of canine herpesvirus by Goodheart and his collaborators indicate that the correct value is 32 \%(Goodheart & Plummer, 1975 and Goodheart, personal communication)].\]
N. M. Wilkie, submitted for publication). Equine abortion virus also seems to share this general arrangement (Fig. 1, Class 2; P. Sheldrick & N. Berthelot, personal communication). In contrast, DNA from channel catfish virus is terminally redundant but has no large internal duplications (P. Sheldrick, N. Berthelot & S. Chousterman, personal communication) and the DNAs from herpesvirus saimiri and herpesvirus ateles each have a long unique sequence of about $70 \times 10^6$ with multiple reiterations of a simple $(G+C)$-rich sequence at their termini (Bornkamm et al. 1976). In these latter three cases it therefore appears that there is a single orientation of unique sequence (Class 1, Fig. 1). It remains to be seen if these interesting differences can provide a useful basis for sub-grouping or whether they are merely samples from a continuum of eccentric arrangements of palindromic and unique base sequences in the DNAs of herpesviruses.

Mean nucleotide composition and intramolecular heterogeneity of nucleotide composition of the DNAs of herpesviruses

The mean nucleotide composition of DNA has long been recognised as a valuable indicator of gross sequence relationships and thus as a parameter of taxonomic value (Sueoka, 1961). For this reason, considerable effort has been expended to obtain values for the nucleotide composition of the DNA from many herpesviruses (Goodheart & Plummer, 1975). Indeed this presently constitutes the only directly comparable measure of diversity which is available for a significant proportion of the present members of the group. We have collected this information in Fig. 2. As has been noted previously (Ludwig, 1972a; Wildy, 1973; Roizman & Furlong, 1974; Goodheart & Plummer, 1975) the values obtained for mean $% (G+C)$ span an unprecedented range from about 33 $% (G+C)$ (canine herpesvirus and cottontail rabbit herpesvirus) to 75 $% (G+C)$ (B-virus) and although the values are not evenly distributed throughout this range the present significance of possible sub-groupings is uncertain. Herpesviruses from a single host are distributed widely throughout the range (see e.g. human viruses indicated by filled square symbols in Fig. 2), and although there is some indication for a clustering of ‘cytomegaloviruses’ from 50 to 60 $% (G+C)$ this may be due as much to a false lumping of these viruses as to the existence of a genuine correlation. Although viruses which appear closely related by other criteria (e.g. cross neutralization and $\geq 10\%$ cross hybridization) have similar $% (G+C)$s not all viruses with similar $% (G+C)$ are closely related by these other criteria. We shall also see that there is evidence for antigenic relationships between members of the group which differ considerably in mean $% (G+C)$.

One further point must be considered before it is possible to evaluate the necessary implications of these large differences in mean nucleotide composition. It is apparent that if the nucleotide composition of each part of the genome was close to that of the whole genome then substantial differences in mean $% (G+C)$ of a pair of genomes would clearly indicate the absence of a significant fraction of common nucleotide sequences. Whilst the redundancy of the genetic code may be invoked to permit non-homologous nucleotide sequences to encode homologous proteins there is little indication for examples of this even on the scale of a single gene let alone a complex genome. Such arguments become unnecessary, however, if cognizance is taken of the existence and extent of intramolecular heterogeneity in the nucleotide composition of these genomes. Gross estimates of such heterogeneity may be obtained from, e.g., the buoyant density distribution of sheared DNA or from transforming the thermal denaturation transition into an expression of the compositional nucleotide distribution. We have added some of this information from published denaturation curves to Fig. 2 (see, e.g., De Ley, 1969 for a more detailed discussion and
application of this approach). The solid bars in Fig. 2 indicate some crude estimates for the 5 to 95% limits of the compositional nucleotide distributions for selected viruses and clearly show that the compositional nucleotide distribution of individual herpesvirus DNAs is remarkably wide. The degree of overlap between these distributions is then the relevant indicator of the theoretical constraints on maximum possible homology between genomes. If the heterogeneity for all members of the group is comparable to those shown it is obvious that there is no theoretical impediment to possession of a significant number of homologous genes by viruses differing in composition by as much as 30% (G+C).

Our enthusiasm for this observation must be tempered as the above treatment merely gives an indication of limits to the maximum possible homology; available estimates of actual homology indicate that very few cases approaching these maxima may be expected. However, the existence of large discrepancies in the nucleotide composition of different segments of herpesvirus genomes also suggests more readily how deletions and duplications of relatively small sections of these DNAs could give rise to rapid changes in the mean % (G+C) of the genome. For example, the removal of reiterated sequences of 70% (G+C) but a kinetic complexity of only ~ 3 × 10^4 from the herpesvirus saimiri genome of 45% (G+C) changes its mean % (G+C) to 35% without a significant loss of genetic information (Fig. 2).

Direct measures of sequence homology

Available measurements of cross hybridization between herpesviruses also contribute as much to separating as they do to allying members of the group. Herpes simplex virus type 1 and 2 show up to 50% homology (Kieff et al. 1972; Ludwig, Biswal & Benyesh-Melnick, 1972; Sugino & Kingsbury, 1976), HSV-1 and bovine mammillitis virus an estimated 14% (Sterz, Ludwig & Rott, 1974) and HSV-1 and pseudorabies a reported 8 to 10% homology (Ludwig et al. 1972). However, HSV-1 shows less than 5% homology with equine coital exanthema virus, which has an almost identical mean % (G+C), varicella-zoster (Ludwig, 1972a, b), equine abortion virus (Ludwig et al. 1971), Epstein–Barr virus, human cytomegalovirus (Huang & Pagano, 1974) and Marek’s disease virus (Bachenheimer et al. 1972). Similarly the catalogue of negatives extends to failures to detect homology between human and simian cytomegaloviruses (Huang & Pagano, 1974), Epstein–Barr virus and Marek’s disease virus (Zur Hausen et al. 1970; Zur Hausen & Schulte-Holthausen, 1970) and between the Lucké herpesvirus and frog virus 4 (Gravell, 1971). The only reported examples of extensive homology other than the HSV-1, HSV-2, bovine mammillitis group (above) are between herpesvirus atetes and herpesvirus saimiri (L-sequences show 35% homology but H-sequences show no significant homology; C. Mulder & B. Fleckenstein, personal communication) and between Epstein–Barr virus, the chimpanzee leukocyte herpesvirus and herpesvirus papio (at least 35% homology; Gerber, Pritchett & Kieff, 1976).

Other measures of homology (e.g. heteroduplex mapping, the number and distribution of common sites of cleavage by restriction endonucleases, and heterospecific recombination) are proving useful for the detailed analysis of close relationships but are probably not suited for detecting small regions of homology as would seem to be required to achieve useful sub-grouping of herpesviruses. Further progress on the direct analysis of sequence homology will therefore require much more careful experimentation to discriminate the genuine absences of homology from all the ≤ 5 to 10% values on which we presently rely. The technical difficulties of measuring imperfect homology over a small fraction of a 100 × 10^6 mol. wt. genome with heterogeneous base composition are obviously considerable. We must therefore see if an analysis of the gene products permits us to detect these limited homologies.
Homologies and analogies in number, size, function and immunological specificity of herpesvirus proteins

Number and nature of structural and non-structural proteins; homologies and analogies between polypeptides

The synthesis of about 50 virus-specific polypeptides with mol. wt. from 15 to > 250000 can be detected by SDS-polyacrylamide gel fractionation of lysates from cells infected with HSV-1 and HSV-2 (Honess & Roizman, 1973; Powell & Courtney, 1975). The sum of the mol. wt. of the novel infected cell polypeptides which have so far been detected can account for about 75% of the anticipated coding capacity of the herpes simplex virus DNA and include numerous non-structural polypeptides as well as structural polypeptides and their precursors (Honess & Roizman, 1973). Careful comparisons of structural and non-structural proteins in cells infected with viruses of the two herpes simplex serotypes shows that although many analogous proteins can be detected the majority of these analogous gene-products differ in mol. wt. (see below). However, analogous polypeptides specified by HSV-1 and HSV-2 may be grouped into similar classes which differ in their relative rates of synthesis at different times throughout the virus growth cycle and some progress has been made in outlining the rudimentary aspects of the control processes which define this temporal sequence of virus protein synthesis (Honess & Roizman, 1974, 1975; Powell & Courtney, 1975). Although aspects of the controls presently known are similar in these two cases there is little reliable comparative data on protein synthesis in cells infected with other members of the group and, in particular, no estimate of differences in the aggregate polypeptide mol. wt. of viruses with apparently lower kinetic complexity (e.g. channel catfish virus). Whilst we would therefore emphasize the importance of studies which analyse differences in the strategy of control processes employed by different members of the group we cannot make many useful remarks on the basis of present information. We can, however, illustrate the potential contribution of such studies by reference to comparative studies on two virus-specified non-structural proteins, DNA polymerase and thymidine kinase and on comparative analyses of polypeptides from purified virus particles of a limited number of herpesviruses.

Despite many claims and counter-claims for numerous virus-specified enzymes in cells infected with herpesviruses rather few have received consistent attention (Keir, 1968). However, some evidence for a virus-specified DNA polymerase has been obtained in cells infected with a variety of herpesviruses and the properties of herpesvirus thymidine kinase have received similar attention. HSV-1 and HSV-2 (Keir et al. 1966; Hay, Moss & Halliburton, 1971), human cytomegalovirus (Huang, 1975a; Hirai, Furukawa & Plotkin, 1976), Epstein-Barr virus (Miller, Glaser & Rapp, 1977), pseudorabies virus (Hamada, Kamiya & Kaplan, 1966; Halliburton & Andrew, 1976), Marek's disease virus (Boezi et al. 1974), the turkey herpesvirus (Leinbach et al. 1976), equine abortion virus and equine coital exanthema virus (Allen, O'Callaghan & Randall, 1977) all specify the production of a novel DNA polymerase activity in infected cells. In most of the above cases the enzyme is distinguished from that of the host by novel immunological specificity, salt stimulation and relative sensitivity to phosphonoacetic acid. The replication of these viruses and, in addition, of herpesvirus saimiri and murine cytomegalovirus has been reported to be sensitive to phosphonoacetic acid (Duff & Overby, 1975; Huang, 1975b). The DNA polymerases of HSV-2 and equine abortion have recently been the subject of purification studies which have shown, somewhat surprisingly, that in the case of both these viruses a single polypeptide of about 140000 mol. wt. is responsible for the in vitro virus-specific DNA polymerase
reaction (Allen et al. 1977; D. Purifoy & K. L. Powell, personal communication). There is some immunological cross reactivity between DNA polymerases of HSV-1 and HSV-2 but not between these enzymes and the pseudorabies DNA polymerase, or between the enzymes of equine abortion and equine coital exanthema virus (Halliburton & Andrew, 1976; Allen et al. 1977). Thus, whilst the specification of a DNA polymerase appears to be a unifying property of the group, these enzymes are immunologically distinct. Sensitivity to phosphonoacetic acid would obviously constitute a simple and useful group-defining characteristic, but there are as yet insufficient careful quantitative comparisons of the effects of the drug on other viruses with virus-specified polymerases (e.g. poxviruses and cytoplasmic deoxyriboviruses). Moreover, resistant mutants of HSV-1 and HSV-2 are sufficiently readily selected (Hay & Subak-Sharpe, 1976; Honess & Watson, 1977) to infer that sensitivity is not an inevitable property of the enzymes of these herpesviruses and unselected isolates of different herpesviruses do not show a uniform sensitivity to the drug (e.g. equine abortion virus is much more sensitive to the drug than is herpes simplex virus; Duff & Overby, 1975). It therefore remains to be seen if differential sensitivity can provide a useful group or sub-group characteristic.

Studies of thymidine kinase activity do suggest some interesting sub-groups with differences in their enzymic repertoire. HSV-1 and HSV-2 both encode immunologically related, but non-identical, polypeptides of 44,000 and 43,400 mol. wt. which bear both thymidine and deoxycytidine kinase activities (Jamieson & Subak-Sharpe, 1974; Thouless & Wildy, 1975). Herpesvirus tamarinus and varicella-zoster virus also produce enzymes with both thymidine and deoxycytidine kinase activities which are not cross-neutralized by antibody to the herpes simplex virus enzyme (Leung et al. 1975; Kit & Dubbs, 1977; Ogino, Otsuka & Takahashi, 1977); in contrast, pseudorabies virus and the herpesvirus of turkeys produce an enzyme which only bears thymidine kinase activity (Jamieson, Gentry & Subak-Sharpe, 1974; Leung et al. 1975) and neither activity appears to be encoded by human cytomegalovirus (Zavada et al. 1976), herpesvirus aotus or equine abortion virus (Leung et al. 1975; Jamieson et al. 1974). Avian infectious laryngotracheitis virus and bovine mammillitis virus also produce a thymidine kinase activity but we have no information on the phosphorylation of deoxycytidine by these enzymes (Kit et al. 1974; R. W. Honess, personal communication). Whilst the presence or absence of thymidine kinase has yet to be correlated convincingly with biological properties of these viruses the function provides a clear illustration of the hazards of assuming that all herpesviruses necessarily follow the same strategy as herpes simplex virus. It also suggests the possibility of sub-grouping viruses on the basis of detailed differences in these strategies. These possibilities and some attendant difficulties may also be illustrated by limited data on virus structural polypeptides.

Spear & Roizman (1972) provided the first thorough examination of the polypeptides of purified enveloped herpes simplex virus type 1 by high-resolution polyacrylamide gel electrophoresis and although subsequent studies have shown the presence of additional minor components and the existence of limited differences in the mol. wt. of structural polypeptides of different isolates of HSV-1 (Heine et al. 1974; Pereira et al. 1976) the descriptions given by Spear & Roizman (1972) of major polypeptides of the enveloped particle and Gibson & Roizman (1972) of the polypeptides of the virus capsid, remain adequate. Given a reliable purification procedure and effective electrophoresis methods it should now be possible to make detailed comparisons of the number and mol. wt. of homologous or analogous components of different viruses. In fact, despite a number of reports of the structural polypeptide composition of individual members of the group relatively few studies have undertaken this type of comparison.
Fig. 3. Diagrammatic comparisons of the number, apparent mol. wt. and relative contribution of major polypeptides from purified enveloped particles of eight herpesviruses. Data in (a) for murine cytomegalovirus (MCMV) are taken from Kim et al. (1976), for human cytomegalovirus isolate AD169 (HCMV) from Fiala et al. (1976) and for Epstein–Barr virus (EBV) from Dolyniuk, Pritchett & Kieff (1976). Part (b) is based on results of Killington et al. (1977), for HSV-1, HSV-2, bovine mammillitis virus (BMV), pseudorabies virus (PRV) and equine abortion virus (EAV) with annotation of major capsid components, 'C', from Gibson & Roizman (1972), Stevely (1975) and Perdue et al. (1974). The polypeptide VP22 of HSV-1 and HSV-2 (annotated 'C' within a lozenge) is the major phosphoprotein of these viruses and these components, as well as glycosylated polypeptides, migrate anomalously in gels of different strengths. Only polypeptides which constitute ≥ 1% of protein mass are shown and solid and cross hatched or stippled bands are used to represent components which are electrophoretically homogeneous or heterogeneous respectively. Most of the heterogeneous components are glycosylated.
Results from one such comparative study of five herpes viruses (Killington et al. 1977) have emphasized the importance of direct comparisons. All the five viruses studied (HSV-1, HSV-2, bovine mammillitis virus, pseudorabies virus and equine abortion virus) had complex patterns with over 16 major polypeptides and at least three complex glycoprotein regions and all had a major high mol. wt. (> 140 000) capsid polypeptide.

Detailed comparison showed that although the mol. wt. of almost every analogous polypeptide differed between HSV-1 and HSV-2 there was an obvious and extensive one-for-one correspondence. Similarly, despite differences in mol. wt. there were many likely analogues of HSV polypeptides in bovine mammillitis virus. However, with few exceptions, it was difficult to recognize analogous components in pseudorabies virus and equine abortion virus either in comparison to each other or to the other three viruses. There was no single polypeptide of identical mol. wt. in all five viruses (Fig. 3 b). In the left-hand panel of Fig. 3(a) we have shown how a published pattern of human cytomegalovirus is substantially different not only from the patterns of Fig. 3(b) but even from that of murine cytomegalovirus, which has some similarities to equine abortion virus. The Epstein–Barr virus also differs from that of human cytomegalovirus and general resemblances can be seen between its pattern and those of equine abortion virus and murine cytomegalovirus.

In that numerical analysis of polypeptide patterns may be impossible over a wide range of herpesviruses, perhaps we should reserve this kind of analysis for more similar patterns for a sub-set of viruses identified as being clustered together by other means, for example, by immunological methods which we shall now consider.

Homologies within polypeptides – common antigens

The taxonomic significance of comparative serology has been noted by Sokal & Sneath (1963) who point out that it can produce a matrix of similarity coefficients analogous to that produced by techniques of numerical taxonomy. Similarly I.C.T.V. (Fenner, 1976) pay serology the compliment of allowing it to determine the extent of the genus herpesvirus (or herpes simplex [? sub-] group) within the herpetoviridae.

A bewildering array of techniques has been used in serological studies of herpesviruses. We shall deal with neutralization and gel immunodiffusion and mention briefly other methods such as complement fixation, immunofluorescence and immune agglutination of virus particles. Current knowledge often relates to studies of small groups of viruses with sufficient common membership to make interlinking of the results of these studies by different workers a real temptation, even although a little thought will frequently show the reagents and methods of the different groups to be incompatible. It is therefore appropriate, when serological information is accumulating, to attempt to survey the situation. While the picture is far from clear there are perhaps sufficient landmarks to allow erection of a few signposts for the way ahead even if only as a safeguard against the day when the acorns of today’s experiments have produced an impenetrable forest.

Neutralization

Neutralization of virus infectivity has been a favoured method in serological work because of its simplicity and sensitivity which allows detection of small differences between two viruses. I.C.T.V. have suggested cross neutralization as the prime serological relationship for qualification as a member of the herpesvirus genus. Such a taxonomic division might be somewhat artificial since, in principle, it could be based on small differences in a single minor gene product. However, as we shall see, cross neutralization seems to correlate with more substantial resemblances.
Results of virus neutralization studies have been presented in a number of ways. Frequently this is done by plaque reduction tests in which the dilution of antiserum which produces a given degree of neutralization (often 50%) is measured. Less often a neutralization rate constant, \( k \), defined as \( (2.303 \times \text{serum dilution in neutralization mixture} \times \log \text{virus survival} + \text{time of reaction}) \), is used. This should be determined from straight line plots of log virus survival against time and/or antibody dilution and if so is to be preferred to other measures in that it can then be assumed to be determined from conditions of antibody excess. Plainly a serum dilution end point from a plaque reduction test should give a directly comparable figure (50% dilution end point = \( k \times \text{time of incubation} + 0.69 \)) assuming that the dilution end point is derived from a straight line plot of serum dilution against log virus survival.

Before discussing the results of comparative neutralization tests it is necessary to indicate the extent of our knowledge or ignorance on the nature of the process to which we are going to assign such weight. Although the precise mechanism of neutralization is not well understood, investigations of the cross neutralization of HSV-1 and HSV-2 have made clear that virus infectivity may be neutralized by attachment of antibody at any one of several different antigenic sites. Following earlier studies (Plummer, 1964; Pauls & Dowdle, 1967) which had shown the two types to be differentiable by the asymmetry of cross neutralization, Sim & Watson (1973) showed that either could be neutralized by antibodies to a common antigenic component, Band II. Antibodies to this component are present in antisera to both viruses. Additionally each could be neutralized by type-specific antibodies identified by the fact that each antiserum would still neutralize the homologous virus after absorption with heterologous antigen to remove cross reacting antibodies; at least two type-specific sites seemed to be involved (Sim & Watson 1973; Powell et al. 1974). Cross-absorption studies with recombinants between HSV-1 and HSV-2 have extended our knowledge of the number of type-specific sites which can interact with neutralizing antibody. For example, if one recombinant possesses type 1-specific sites A, B, C and another A, B, D then type 1-specific antiserum absorbed with the first will neutralize the second and vice versa. By experiments of this kind Halliburton et al. (1977) recognized a minimum of four type 1-specific sites and three type 2-specific sites. Even this figure for type 1-specific sites has been increased by studies on the heterogeneity of individual HSV-1 strains (R. A. Killington, E. J. Ouldridge & D. H. Watson, personal communication); reciprocal absorption experiments of the kind described above, using different strains, have identified additional sites.

These studies on the number and nature of antigenic sites reacting with neutralizing antibodies have emphasized the diversity of herpes simplex virus – not only intertypically but also apparently intratypically – they have also indicated a degree of unity between the two types mediated by the Band II site. Cross neutralization may usefully be expressed as a ratio of homologous and heterologous \( k \) values (or serum end point dilutions) with antiserum to one virus. Ideally we should like to take a number of antisera to individual components and study their reactions with a number of herpesviruses. It is possible that even where these show a reaction with antigenically shared components that the \( k \) ratio will show asymmetry. For example, antiserum to Band II from type 1 infected cells has a ratio \( k_{\text{HSV-1}}/k_{\text{HSV-2}} \) varying between 2 and 5, and this may represent a molecular difference between polypeptides which nevertheless share an antigen site (the Band II polypeptides of HSV-1 and HSV-2 differ in apparent mol. wt.: R. E. Randall, personal communication) or the possibility that antibodies reacting with identical molecules on the two viruses may exert their neutralizing effect by a second order (e.g. steric) effect on the surrounding non-identical regions of the virus particle. In practice, of course, we find that cross reactions
Table I. Cross neutralization of herpesviruses

<table>
<thead>
<tr>
<th>Virus 1</th>
<th>Virus 2</th>
<th>k ratio*</th>
<th>Immunological† distance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1, HSV-2</td>
<td></td>
<td>6·8, 2·1</td>
<td>3·8</td>
<td>Killington et al. (1977)</td>
</tr>
<tr>
<td>HSV-1, SA8</td>
<td></td>
<td>128, 4</td>
<td>22·4</td>
<td>Hull (1973)</td>
</tr>
<tr>
<td>HSV-1, B-virus</td>
<td></td>
<td>8, 8</td>
<td>8</td>
<td>Stevens et al. (1968)</td>
</tr>
<tr>
<td>HSV-1, canine herpes</td>
<td></td>
<td>44, 4</td>
<td>13</td>
<td>Watson et al. (1967)</td>
</tr>
<tr>
<td>SA8, B-virus</td>
<td></td>
<td>8·2, 2·5</td>
<td>4·5</td>
<td>Aurelian (1968)</td>
</tr>
<tr>
<td>HSV-1, bovine mammillitis virus</td>
<td></td>
<td>8, 4</td>
<td>5·6</td>
<td>Watson et al. (1973)</td>
</tr>
<tr>
<td>HSV-1, infectious bovine rhinotracheitis</td>
<td></td>
<td>60, 4</td>
<td>15·8</td>
<td>Killington et al. (1977)</td>
</tr>
<tr>
<td>HSV-1, herpesvirus tamarinus</td>
<td></td>
<td>&gt; 256, &gt; 256</td>
<td>&gt; 256</td>
<td>Stevens et al. (1968)</td>
</tr>
<tr>
<td>HSV-1, equine abortion virus</td>
<td></td>
<td>128, 32</td>
<td>64</td>
<td>Blue &amp; Plummer (1973)</td>
</tr>
<tr>
<td>HSV-1, pseudorabies virus</td>
<td></td>
<td>&gt; 3600, &gt; 14</td>
<td>&gt; 220</td>
<td>Killington et al. (1977)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>64, 64</td>
<td>64</td>
<td>Blue &amp; Plummer (1973)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 3600, &gt; 888</td>
<td>&gt; 1800</td>
<td>Killington et al. (1977)</td>
</tr>
</tbody>
</table>

* For each pair of viruses the first ratio quoted is $k_{\text{homologous}}/k_{\text{heterologous}}$ for antiserum to the first named virus and the second ratio is the corresponding figure derived from antiserum to the second virus.

† Geometric mean of two k ratios.

Table I we have listed figures for the observed cross reactions between a number of pairs of herpesviruses. The results are expressed as $k_{\text{homologous}}/k_{\text{heterologous}}$ for each pair, values being given for the ratio for the antiserum to each virus. The Table also shows the geometric mean of the two ratios, as used by Chu, Andrews & Gledhill (1950) to combine reciprocal antibody titres and defined as immunological distance by Mainardi (1959). The Table illustrates marked asymmetry in the ratios observed with certain pairs of antisera as well as variations between ratios derived by different workers. Despite this HSV-1, HSV-2, SA8, B-virus, bovine mammillitis virus and canine herpes virus are fairly closely linked by the cross neutralization data. Infectious bovine rhinotracheitis and herpesvirus tamarinus are only marginally linked to HSV-1, while equine abortion virus and pseudorabies virus, which exhibit no significant cross neutralization with HSV-1, provide limiting values for a distance representing no relationship. It will be noted here that the ratio for antiserum to equine abortion virus is expressed as $> 14$, a reflection of the poor neutralizing activity of the antiserum against the homologous virus.

We have already indicated that the cross neutralization of HSV-1 and HSV-2 is mediated by antiserum to Band II and we may enquire if this is a common antigenic component of all the viruses shown to be linked to HSV-1 by cross neutralization. Certainly antiserum to Band II neutralizes bovine mammillitis virus (Killington et al. 1977) and Band II antigen inhibits the neutralization of HSV-1 by antiserum to B-virus (Caroline Sim & D. H. Watson, personal communication). It is still not clear, however, that this is the sole component involved in all the cross neutralizations and some preliminary evidence suggests that an additional antigenic site involved in cross neutralization of HSV-1 and HSV-2 may not be present on bovine mammillitis virus (Jane Yeo & R. A. Killington, personal communication).

We have been concerned here with delineation of diversity between species and it is
important to be sure that this differs in amount if not in kind from ‘intraspecies’ differences. We have already indicated that HSV-I strains themselves show some diversity. However, the evidence shows that the greatest immunological distance among the strains studied by reactions with general antiserum is about 1.5, much closer than any of the ‘interspecies’ differences indicated in Table I. This is in clear distinction from the results with a series of isolates all termed equine herpes 2 (equine cytomegaloviruses). Plummer, Gocdheart & Studdert (1973) show homologous/heterologous ratios up to > 512 using antisera to four of these viruses in neutralization tests of these four strains and six others. For the four strains for which reciprocal antisera data are available immunological distances of 10, 16, 32 and 128 may be calculated (reciprocal titres are not available for the strains giving the highest homologous/heterologous ratios). Plainly some of these strains are as diverse as many of the different species of Table I and indeed some of the ratios recorded (e.g. > 512 with no significant neutralization observed) would, on the basis of the data in Table 1, suggest a virus of a different ‘genus’ if cross neutralization is to be taken as a genus limiting measure as suggested by I.C.T.V. These observations suggest a need to exercise caution in the use of the phrase ‘serologically identical’. Plainly, immunological differences of the order quoted should indicate the inappropriateness of such a description. However, we have noted that strains of HSV-1 are not ‘serologically identical’ although the immunological distances, measured by general antiserum reactions, are small. These distances are increased if type-specific antisera are used and become infinite if strain-specific sera, derived by reciprocal absorption, are used. If differences cannot be revealed by such absorption tests, ‘serologically identical’ may be used with the reservation that the right differentiating test may not have been done.

If Table I defines one ‘genus’, although with a rather fuzzy boundary, we may enquire if others can be similarly defined from data on other viruses. Hull (1973) suggests that herpesvirus tamarinus and spider monkey virus cross neutralize and that they do not cross neutralize HSV-I (Table I suggests tamarinus is only a marginal member of the herpes simplex group or ‘genus’). These two would therefore perhaps form the nucleus of another ‘genus’ for a cluster of viruses defined in this way. We prefer to call a cluster of viruses defined by significant serological relationships, a ‘seron’. Where, as here, the cluster is defined by the more specific serological relationship of cross neutralization we term the cluster so defined a ‘neutroseron’.

So far then we have defined two possible neutroserons. Four others are EB virus, herpesvirus papio and a chimpanzee agent present in leukocyte cell lines (Gerber et al. 1976); Marek’s disease virus and turkey herpesvirus (Witter et al. 1970); herpesvirus atelefs and herpesvirus saimiri (Deinhardt, 1973); varicella-zoster virus and several viruses causing varicella-like diseases of non-human primates (Felsenfeld & Schmidt, 1977).

Other serological tests

Cross reactions between herpesviruses have also been studied by complement fixation, immunofluorescence, agar gel immunodiffusion and in a few instances by observing immune agglutination of virus particles in the electron microscope.

Complement fixation has the advantage of simplicity and sensitivity and is capable of supplying quantitative data. The interpretation of these, however, may be somewhat difficult. Not only may the homologous reaction be with a different component to that studied in the heterologous but the antiserum titres quoted for homologous and heterologous reactions may depend on whether they are obtained for comparable concentrations of the two antigen preparations. As with neutralization reactions a limit of homologous/hetero-
Table 2. Viruses serologically related to HSV-1 by methods other than neutralization

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Complement fixation</th>
<th>Gel diffusion</th>
<th>Immunofluorescence</th>
<th>Particle agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-2</td>
<td>(2)</td>
<td>(1)</td>
<td>(3)</td>
<td>(1) (2)</td>
</tr>
<tr>
<td>B-virus</td>
<td></td>
<td>(4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine mamillilitis</td>
<td>(5)</td>
<td>(6)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Infective bovine rhinotracheitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equine abortion</td>
<td>(3)</td>
<td>(6)</td>
<td>(7)</td>
<td></td>
</tr>
<tr>
<td>Herpesvirus tamarinus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudorabies</td>
<td>(4)</td>
<td>(2)</td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td>EB</td>
<td></td>
<td>(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lucké</td>
<td></td>
<td>(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>(8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marek’s disease</td>
<td>(8)</td>
<td></td>
<td>(2)</td>
<td>(2)</td>
</tr>
<tr>
<td>Turkey herpesvirus</td>
<td></td>
<td></td>
<td>(2)</td>
<td>(2)</td>
</tr>
<tr>
<td>Varicella-zoster</td>
<td>(9)</td>
<td></td>
<td></td>
<td>(1)</td>
</tr>
</tbody>
</table>

(1) Honess et al. (1974); (2) Ross, Frazier & Biggs (1972); (3) Plummer (1964); (4) Watson et al. (1967); (5) Sterz et al. (1974); (6) Killington et al. (1977); (7) Blue & Plummer (1973); (8) Kirkwood, Geering & Old (1972); (9) Caunt & Shaw (1969).

Logous titres for negative reactions is needed for a full analysis and this may not be easily acquired because the level of non-specific reactions may obscure slight specific reactions. In principle, however, the method should be capable of yielding a series of immunological distances capable of analysis by the methods of numerical taxonomy. We have not attempted such an analysis here because the varied sources of published data make the comparisons difficult.

Immunofluorescence is plainly very sensitive but very dependent on qualitative judgement, making ‘end point’ antiserum titres difficult to interpret.

Agar gel immunodiffusion tests can give estimates of the number of common components which may be significant if derived from studies in which several antigen dilutions are tested against a range of antiserum dilutions as can be done by an appropriate arrangement of well patterns (Killington et al. 1977). Ideally one would wish to test antisera against specific components with antigen preparations from different viruses and obtain quantitative data on dilution end points etc.

Particle agglutination is not a particularly sensitive method and ideally requires purified preparation of virus. Its main use is in revealing topological locations of cross reacting component on, e.g., capsids or envelopes. Thus HSV-1 and HSV-2 have cross reacting components on both envelope and nucleocapsid surfaces, HSV-1 and pseudorabies have such components on nucleocapsids only (Honess et al. 1974).

To give an idea of the extent of the potential seron of herpesviruses between which serological reactions have been defined we summarize in Table 2 data on cross reactions between HSV-1 and other herpesviruses. The Table gives only a representative selection of publications recording the cross reactions listed. Reciprocal reactions have been noted between many of the viruses listed but have not been shown in the interests of simplicity. However, in some cases viruses not listed have been reported to cross react with viruses on the list although not with HSV-1, and such viruses would then be potential candidates for a seron defined as including all viruses which have been serologically linked at any level with any other virus already included in the seron. Thus, simian cytomegaloviruses have been
Table 3. The number of precipitin bands formed in agar gel immunodiffusion reactions of antisera to herpesvirus against antigens of cells infected with homologous and heterologous viruses

<table>
<thead>
<tr>
<th>Antiserum prepared against</th>
<th>Number of precipitin bands observed against extracts of cells infected with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HSV-1</td>
</tr>
<tr>
<td>HSV-1</td>
<td>$\geq 12$</td>
</tr>
<tr>
<td>HSV-2</td>
<td>$\geq 5$</td>
</tr>
<tr>
<td>BMV</td>
<td>2</td>
</tr>
<tr>
<td>EAV</td>
<td>0</td>
</tr>
<tr>
<td>PRV</td>
<td>2</td>
</tr>
</tbody>
</table>

claimed to cross react in complement fixation tests with human cytomegaloviruses (Black, Hartley & Rowe, 1963; Dreesman & Benyesh-Melnick 1967). Similar relationships have been quoted between EB virus, herpesvirus papio and chimpanzee leukocyte herpesvirus(es) (Gerber et al. 1976). Linking of viruses to the seron by such second order relationships is, of course, questionable. The data on antigenic drift of influenza viruses emphasize that it is wrong to assume that because A is serologically related to B, which is similarly linked to C, then C must perforce be linked to A. Meaningful linking of viruses can only be done by a fully orthogonal analysis.

In any event, while assembly of such a seron is of some interest its taxonomic significance may be questioned. While we are still far from having demonstrated a herpesvirus group antigen, the odds against the existence of such a component may be justifiably claimed to have declined recently, although we may note that the increasing numbers of serologically related herpesviruses have not brought us much nearer to showing a universal group antigen because new members are being added to the group at almost the same rate as relationships are being shown between older members. If a group antigen were shown, all members of the group would belong to one seron with a minimum entrance qualification, invalidating it as a useful taxonomic sub-division of the group. Plainly we need to be able to identify serons whose members are linked at a defined degree of similarity, either by using cross neutralization as an entry qualification (i.e. using the neutroseron as the taxonomic division) or by an analysis of other serological data by numerical taxonomy.

As an outline of this kind of approach we wish to analyse the results of Killington et al. (1977) on numbers of precipitin bands observed in reciprocal agar gel immunodiffusion tests with five herpesviruses (Table 3). Inspection shows us that HSV-1, HSV-2 and bovine mammillitis virus (already linked in a neutroseron) are more extensively linked than the other two viruses shown. We wish to give some quantitative expression to this. Reciprocal serological data of this kind provide a means of producing a matrix of similarity indices. The data of Killington et al. (1977) indicated that where an antiserum gave a lower number of precipitin bands with one virus than with another the first was a sub-set of the second. Thus, antiserum to HSV-1 when diffused against HSV-2 gave 6 of the 12 precipitin bands given with HSV-1, indicating a cross reaction index of $6/12 = 0.5$. A second estimate of the cross reaction index from the reciprocal reaction is $5/7 = 0.7$ and we use the mean figure of 0.6 as an estimate of the cross reaction index between HSV-1 and HSV-2. If such indices are calculated for each pair of viruses they may be clustered by a weighted pair group method (Sokal & Sneath, 1963), giving the dendrogram of Fig. 4. This tells us what we already surmised from inspection, that HSV-1 and HSV-2 are the most closely linked pair, with BMV being linked to this pair and EAV and PRV linked at a considerably lower
level. Finally, the whole group is linked at very low level. Serons may now be defined at varying quantitative levels of cross reactions. Thus we might decide that only clusters linked at an index of ≥ 0.5 would be defined as a seron. On this basis the viruses HSV-1, HSV-2, BMV (already identified as members of a neutroseron) would form a seron which would exclude EAV and PRV which would not themselves form a seron. On the other hand a limit of ≥ 0.25 would form a second seron from these two viruses. To include all the viruses would need a limit of ≥ 0.075.

It would of course be unreasonable to decide from an analysis of only five viruses whether the 0.5 seron (HSV-1 + HSV-2 + BMV), or the 0.25 serons (HSV-1 + HSV-2 + BMV and EAV + PRV) are the most apt although we might conclude that the 0.075 seron necessary to include all the viruses is at a low level of relatedness. Our point is only to indicate how a more comprehensive analysis might be achieved. This would then reveal an appropriate level for delineation of serons. Plainly such an analysis requires an experimental comparison on a larger scale which would be most usefully achieved by collaborative effort by an exchange of sera and standardization of tests by, for example, use of a gel pattern which allows testing of antigen at high concentration with antisera of comparable quality.

DISCUSSION

Throughout this survey we have sought measures of similarity and difference between herpesviruses; first at the level of the host and disease and the gross properties of size, sequence arrangement and base composition of the genome and, finally, the number and properties of some gene products. We now wish to see how far these measures are correlated to give a coherent expression of unity and diversity. First of all, the essential unity of the family is confirmed by the common possession of DNA with single strand interruptions typical of such viruses.
and a mol. wt. > $80 \times 10^6$, a major capsid polypeptide mol. wt. > 140,000, a virus-specific non-structural DNA polymerase and a propensity for persistence in the absence of overt disease.

Proceeding to diversity we recall that although any property may be used as a basis for classification or grouping...The ends of scientific classification are best answered when the objects are formed into groups respecting which a greater number of general propositions can be made, and those propositions more important, than could be made respecting any other groups into which the same things could be distributed’ (Mill, 1881). We began with what we admitted to be a subjective division on the basis of disease. We can now see that the cytomegaloviruses do not hybridize with viruses of any other group, are not in the same neutroseron and within the group only base composition seems to link them. Certain T cell lymphotropic viruses are linked by cross hybridization data and similar genome arrangement; B cell lymphotropic viruses form a neutroseron and cross hybridize. None of these viruses cross hybridize with viruses of other disease groups.

Viruses of the third group are represented in three of our possible neutroserons (HSV-1 etc.; varicella zoster etc.; herpesvirus tamarinus and spider monkey herpesviruses) with infectious bovine rhinotracheitis as a fringe member of the first and pseudorabies plainly belonging to a distinct, as yet unspecified neutroseron. Perhaps more importantly no members of these neutroserons belong to different disease groups. This group also illustrates that % (G+C) does not correlate with other properties. Virtually the whole range of observed values in Fig. 2 is represented within the group although this range includes groups 1 and 2 viruses. Further, % (G+C) does not correlate exclusively with neutroseron assignment. With the notable exception of canine herpesvirus, all the members of the HSV-1 neutroseron have % (G+C) in the range 64% to 75% but this range includes equine coital exanthema and pseudorabies virus which are clearly not members of the HSV-1 neutroseron.

Conversely, assignment to the HSV-1 neutroseron correlates with similarities in number and mol. wt. of structural polypeptides and with possession of a virus-specific deoxy- pyrimidine kinase. It will be evident that cross neutralization correlates well with several other properties and we have already noted that it correlates well with serological analysis based on more generalized reactions. Paradoxically, a family which may be defined on the basis of morphology alone may perhaps be subdivided on the basis of cross neutralization alone; although intuitively we would reject taxonomy on the basis of such limited criteria, in both cases the correlation with other measures is better than we feel we have any right to expect.

It would appear that systematic taxonomy of the family could proceed by extending to other viruses I.C.T.V.’s policy of defining a HSV-1 genus by cross neutralizability. However we feel that despite our optimistic conclusions on the merit of cross neutralization as a criterion we should await a more complete proof of its taxonomic significance from more extended comparisons with other properties than is at present available. We have dealt with a not necessarily representative minority of herpesviruses and it is obvious that only one of our six possible neutroserons is well characterized or contains a number of viruses. In that it is plainly I.C.T.V.’s intention to give formal names on basis of genus assignment it would be unfortunate if precipitate assignment produced the sort of problem created in the bacterial world where re-assignment has led, for example, to the Corynebacterium acnes of yesterday now being labelled Propionibacterium acnes, P. avidum or P. granulosum. We believe that a systematic taxonomic division should be derived by numerical analysis of a number of criteria. It should be possible, for example, to code measures such as % (G+C),
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genome arrangement, DNA mol. wt. and analyse the results by the methods outlined by Sokal & Sneath (1963).

The trivial point of virus names leads us to note that I.C.T.V.'s current proposal that the HSV-1 group or sub-group be named the 'herpesvirus genus' creates a difficulty in naming other genera. Inelegant as the generic prefixes of the adenoviridae may be, a family name of herpesviridae would have allowed generic names of herpesvirus genus etc.

Recollecting that the aim of I.C.T.V. is to work towards a Latinized binomial nomenclature we recall that it has been said that an appropriate Latin binomial motto for I.C.T.V. would be festina lente. Remembering the origin of the name herpesvirus a Greek binomial motto may be appropriate – ἔσπειρε βραδύτερον.

We should like to thank our colleagues in Leeds (R. A. Killington, R. E. Randall, Elizabeth Ouldridge and Jane Yeo) and elsewhere (J. B. Clements, B. Fleckenstein, G. Goodheart, H. Ludvig, C. Mulder, D. Powell, K. L. Powell, D. Purifoy, B. Roizman, P. Sheldrick and N. Wilkie) for communicating results which are as yet unpublished. In particular we thank Dr P. Sheldrick for providing us with an account of his results and ideas concerning the structure of DNAs from equine abortion virus and channel catfish virus, D. Powell, N. Wilkie and J. B. Clements for a preview of their results with pseudorabies virus DNA and B. Roizman for informing us of the preliminary results obtained by his group with the DNA of bovine mammillitis virus. Professor W. G. Arnott very kindly remedied the deficiencies in our classical education.

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