MOLECULAR EPIDEMIOLOGY OF HERPES SIMPLEX VIRUS INFECTIONS

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PUBLICATIONS AND PRESENTATIONS

The results of some of the work incorporated in this thesis have already been reported jointly with other research colleagues.

Publications

Smith IW, Barr BBB, Slatford K, Robertson D. 1985. Restriction enzyme analysisof HSV isolates from known contacts of patients with genital herpes. Lancet i: 979.

Presentations

(1) Eighth International Herpesvirus Workshop, Oxford, 1983 (Abstract 218).

(2) Scottish Virology Group, Glasgow, June 1984.

(3) Society for General Microbiology, Glasgow, June 1985.

(4) International Society for Sexually Transmitted Disease Research, Brighton, July 1985.

DECLARATION

The investigations and procedures, other than stated, were performed by the author. The thesis is the sole work of the author.

ABSTRACT

The aim of this study was two-fold, (i) to examine variation in the DNA of HSV isolates from patients presenting at the Genitourinary Medicine Clinics and the Nuffield Transplant Unit, Edinburgh, and (ii) to assess the reproducibility, specificity and sensitivity of non-radiolabelled nucleic acid hybridisation systems.

During the 19 month period 1.1.82 - 31.7.83, 224 herpes simplex viruses (180 HSV 2, 44 HSV 1) were isolated from new or returned new patients presenting at the Department of Genitourinary Medicine, the Royal Infirmary, Edinburgh. The non-radiolabelled DNA from all isolates was purified by sodium iodide density gradient centrifugation before being subjected to restriction endonuclease digestion and separation by agarose gel electrophoresis. The analysis was carried out with five restriction enzymes (*EcoRI*, *BglII*, *HindIII*, *KpnI*, *BamHI*) for which published genomic maps are available.

Although the HSV 2 genome was found to be highly conserved, the 180 HSV 2 isolates could be divided into 18 groups on the basis of deletions or additions of specific enzyme recognition sites. In total 9 major variations were detected, three of which have not previously been reported. These sites have been confirmed by nick-translation and hybridisation with ^{32}P-labelled DNA probes. By comparison all 44 HSV 1 isolates were distinguished purely on the basis of major variations, 21 of which were recorded.

The map positions of the major variations of HSV 1 and HSV 2 isolates were not found to correlate with the site of infection, sex, or time of isolation.

The use of mobility variations as a means of determining epidemiological relatedness was examined both on agarose and polyacrylamide gels using ethidium bromide and an ultrasensitive silver stain respectively. Selected HSV DNA preparations were examined to determine the extent of electrophoretic variation. These included 35 concurrent HSV isolates from 17 patients, 14 sequential genital HSV isolates (from 7 patients), 54 HSV isolates from known sexual consorts, 44 sequential oral HSV 1 isolates from 16 renal transplant recipients and 9 HSV 1 isolates involved in possible hospital outbreaks.

Variations were observed mainly in the junction and terminal fragments of the genome, however unique fragments, from both the long and short segments, were observed to vary. These results confirm that mobility variations should not be used as a means of epidemiological distinction. Examination of 54 viruses from known sexual consorts also highlighted the need for caution in the interpretation of RE results, particularly in the absence of clinical and serological data.

Two non-radiolabelled detection systems involving biotin and photobiotin were investigated with a view to establishing their suitability in routine clinical procedures. Both systems were compared for sensitivity and specificity, however several problems encountered with the biotin labelled system prevented an accurate

assessment of its sensitivity. No such problems were encountered with the photobiotin system, which proved reliable, specific and sensitive enabling the detection of nanogram and picogram quantities of HPV, CMV, HBV and HSV DNA.

INTRODUCTION

GENERAL INTRODUCTION

Until the discovery of the acquired immunodeficiency syndrome (AIDS), herpes simplex virus (HSV) infection was perhaps the most widely publicised sexually transmitted disease in the past decade. Recent figures from the USA indicate that HSV still causes major problems for the public health program, being responsible for an excess of 500,000 new cases of genital herpes in 1985. The 'true prevalence' of the virus however, is estimated to be much higher, affecting approximately 20-60% of all Americans (ASM News, 1986).

Historical Background

Herpes febrilis, the common cold sore, was first documented in 100 AD when the term 'herpes' was used to describe a variety of spreading cutaneous lesions (Hutfield, 1966). With time, clinical and epidemiological features gave rise to the suggestion that a different aetiologic agent was responsible for herpes febrilis and herpes genitalis. Nevertheless, no virological distinction was proven until the laboratory identification of two virus serotypes (HSV 1 and HSV 2) by Schneweiss in 1962. Subsequent studies in 1967 (Dowdle *et al.*, 1967; Schneweiss, 1967) correlated the antigenicity of the virus with the anatomical site of isolation, thus giving rise to the association of HSV 1 with herpetic infections above the waist and HSV 2 with genital infections. Current figures now indicate that over 20% of genital herpes is caused by HSV 1.

Epidemiology and Incidence of Herpetic Infections

There has been widespread documentation of the prevalence of HSV infections among various populations and age groups (Smith *et al.*, 1967; Nahmias and Roizman, 1973). In the adult population, a high proportion of individuals have neutralising antibodies to HSV 1, particularly among lower socioeconomic groups. In newborn children transplacental transfer of HSV antibodies may occur, although the antibody levels tend to decline gradually over a 6-8 month period (Robertson *et al.*, 1980). Antibodies to HSV 2 are not usually detected until puberty and are correspondingly associated with a history of sexual activity (Corey and Spear, 1986a).

Incidence of genital herpes in the UK and USA

Data on the incidence of genital HSV in the UK and USA (Fig 1) have shown a marked increase in the rate of HSV infections. In the USA the number of reported cases of genital HSV infections increased dramatically during the years 1974-78. Although a small decline was observed the following year (1979), the rapid increase of HSV infection continued in 1980. Similarly in the UK, from 1971-78, a marked increase in clinically apparent genital herpes was observed.

Prevalence of HSV infections in the UK

(i) England and Wales

Despite the fact that a 50% rise in the total number of HSV isolations was recorded for the periods 1981-85 (Fig 2), the overall increase was due to the rising incidence of genital herpes. In

Figure 1

The incidence of genital herpes simplex virus in the USA and UK (adapted from Becker and Nahmias, 1985)

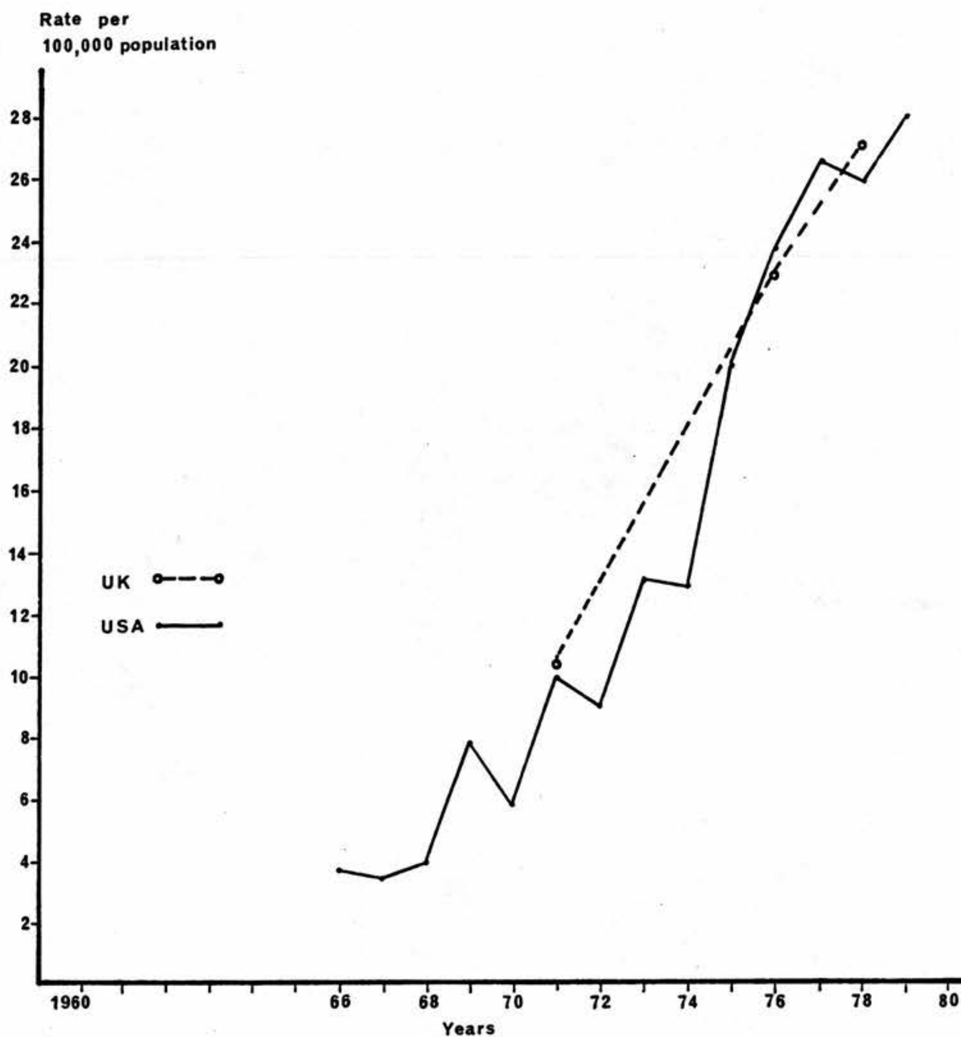
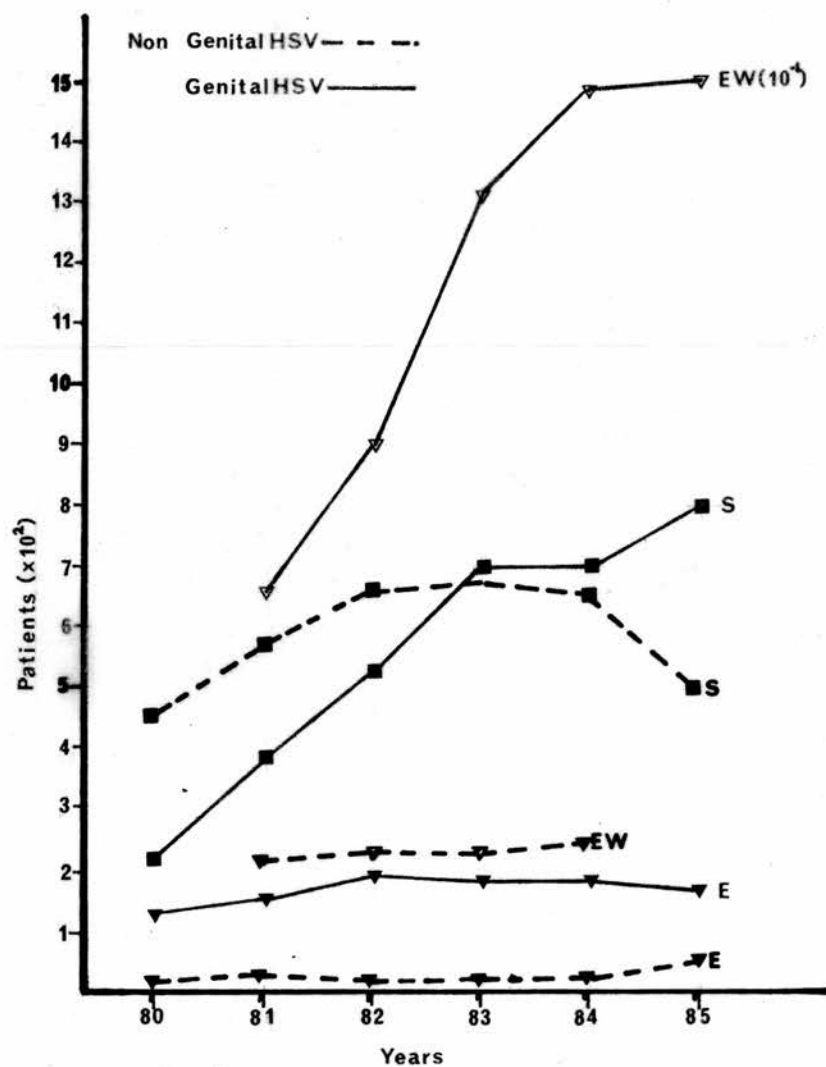


Figure 2

The prevalence of herpes simplex virus infections in the UK.



E – England
W – Wales
S – Scotland

1981, 6660 cases of genital HSV were reported by the laboratories, whereas four years later the number of cases had risen to 15,316 (Source - Communicable Diseases Surveillance Centre).

(ii) Scotland

During the period 1980-85 the total number of cases of herpetic infections investigated by laboratories in Scotland increased from 755 to 1452. Surprisingly, the years 1983-84 showed no apparent rise in the number of genital isolates whereas in 1985 non-genital HSV infections reported by the laboratories decreased by well over 100 cases to 539 (Source - Communicable Diseases (Scotland) Unit).

(iii) Edinburgh

A marked increase in the incidence of genital HSV infections was observed between 1980-82, but there may now be a downward trend (Fig 2). By comparison the number of reported cases of non-genital HSV infections have shown very little variation between 1980-85 (Source - University of Edinburgh).

The Pathogenesis of Herpetic Infections

The first infection with HSV may or may not produce clinically apparent signs and symptoms of disease. Following the first infection, antibodies appear and the virus, unable to be eliminated from the body, enters a latent state. Periodically, reactivations of the latent virus may occur giving rise to virus shedding, either with or without the appearance of localised lesions.

Although no uniform terminology exists to identify the various types of infection, the following definitions are widely accepted:

(a) Primary Infections - infections occurring in individuals without antibodies to the virus.

(b) Initial Infections - the first infection of an individual with a heterologous virus serotype.

(c) Recurrent Infections - infections produced by reactivation of the virus from a latent state.

In general any illness associated with recurrent or initial infection tends to be milder than on primary exposure, due to the presence of antibodies. In some immunosuppressed individuals however, the illness associated with recurrent infection can be severe and in extreme cases fatal, despite the presence of antibodies. Individuals particularly at risk are the newborn, burns patients and organ transplant recipients.

The Mechanisms of Latent Herpes Simplex Virus Infections

It is generally accepted that during the acute phase of infection, herpes simplex virus is taken up by the nerve endings and travels, probably as a non-infectious nucleo-capsid, towards the neurones of sensory ganglia (Klein 1985). When the human host has recovered from the primary infection, the virus becomes established in a latent form in the neurons of the sensory ganglia and may be reactivated by a number of trigger mechanisms (Docherty and Chopan 1974).

As virus can be isolated from the saliva, tears, and genital secretions of apparently asymptomatic individuals (Buddingh *et al*., 1953; Kaufman *et al*., 1967; Jeansson and Molin, 1970), one suggested mechanism of latency is that during a primary infection, the virus may become established in the tissue (eg cervix, salivary or lacrimal gland) close to the site of recurrence, then replicate slowly, until the introduction of a stimulating factor (ie fever, trauma, UV light) makes the area susceptible to localised reinfection (Docherty and Chopan, 1974).

An alternative hypothesis maintains that the virus enters a non-replicative (static) state between reactivations, and may subsequently be activated to a replicative state with, or without, the redevelopment of herpetic lesions (Docherty and Chopan, 1974). In support of this theory is the isolation of HSV from cultures of human ganglia (Baringer and Swoveland, 1973).

Recent findings suggest that the distinction between a 'static' and a 'dynamic' state of latency is by no means clear-cut (Klein, 1985), indeed the processes are most probably not exclusive of one another. However, regardless of the mechanisms of latency, the frequency of reactivating events is determined mainly by the immunological state of the host (Docherty and Chopan, 1974). A notable example of this can be found in individuals with impaired cell-mediated immunity such as organ transplant recipients, in whom therapeutic immunosuppression is usually followed by the development of recurrences.

CLINICAL FEATURES OF HERPETIC INFECTIONS

The following sections provide a brief account of the main clinical aspects for a variety of herpetic infections. The sections dealing with the genitalia and infections in immunosuppressed individuals are more extensive, as these topics are relevant to the present study.

(i) Oral Infections

In young children, from 1-4 years old, HSV 1 is usually the cause of a primary infection in the form of acute gingivostomatitis. The clinical features include the presence of vesicles, which rupture to form ulcers on the tongue, palate and buccal mucosa. However, accompanying features such as fever, malaise, tenderness and loss of appetite are also common. Identical clinical features may be seen in young adults who, having escaped childhood infection, acquire a primary infection from oral contact with someone who has HSV (Robertson *et al*., 1980).

Herpes labialis is usually caused by recurrent HSV 1 infections. The characteristic lesions or "cold sores" develop on, or near, the lips and are often preceded by a burning or tingling sensation. Unlike gingivostomatitis, a high incidence of herpes labialis is found among the older population (Wolonitis and Jeansson, 1977).

(ii) Dermatologic Lesions

(a) A number of cases of traumatic herpes have been reported in sportsmen who practise contact sports and in nail-biting individuals. In such cases the skin lesions do not usually produce scarring, however the recurrent nature of the infection may produce a cosmetic problem for the individual (Hale *et al.*, 1963; Wheeler and Cabaniss, 1965).

(b) Members of several professions including doctors, nurses and dentists are particularly prone to the development of a painful HSV infection of the finger known as herpetic whitlow. Inoculation may occur by direct entry through minor abrasions, or by infection from patients with primary or recurrent herpes (Wheeler and Cabaniss 1965). The onset is usually abrupt with swelling, redness and tenderness at the site of infection.

The infection may either be primary or recurrent, dependent on the presence or absence of antibodies in the infected individual. In either case, the whitlow is usually resolved within two to three weeks (Manzella *et al.*, 1984) in the absence of secondary bacterial infection. Transmission of the virus may occur before healing and has been known to cause outbreaks of gingivostomatitis in patients attending infected dental personnel (Watkinson, 1982; Manzella *et al.*, 1984).

(c) Primary dermal herpes can be experienced both in children and adults, resulting mainly in a few scattered lesions. In some individuals, with complicated eczema, very extensive skin

involvement may be seen in the form of eczema herpeticum, a variety of Kaposi's varicelliform eruption (Feldman and Sonnabend, 1979).

(iii) Ocular Infections

The eyes are particularly vulnerable to primary infection by autoinoculation with HSV 1 from infected hands or saliva. In most cases primary infections are rarely severe (Darougar et al., 1985). However, recurrent infections usually result in acute keratitis that may cause impairment of vision, or in extreme cases, blindness (Kaufman, 1981).

(iv) HSV Encephalitis

HSV infections of the central nervous system (CNS) (known to occur in approximately one in every 500,000 Americans each year) are usually the consequence of herpes encephalitis (Whitley, 1984). Originally HSV encephalitis was thought to be a disease of the elderly. However, figures now indicate that over one third of the cases are found in young children and adolescents (Whitley et al., 1981). In newborns 80% of the infections are caused by HSV 2, whereas HSV 1 infections are more common in the older population (97%) (Whitley, 1984).

The infection becomes localised in the temporal and orbito-frontal areas of the brain, usually resulting in haemorrhagic necrosis. Fever, autonomic nervous dysfunction, seizures and fluctuating levels of consciousness are not uncommon features. In

young children malaise, disorientation and irritability may also occur.

(v) Neonatal Infection

Neonatal HSV infection is often symptomatic, occurring either as a disseminated, or localised disease, dependent on the extent of involvement of the infected infant (Nahmias and Roizman, 1973). Disseminated infections commence ten to twelve days after birth and are combined with signs such as irritability, seizures, jaundice, vesicular rashes and respiratory distress. Eighty per cent of all cases have CNS involvement, although in the majority of cases death is attributed to pulmonary disease (Whitley, 1984).

Both asymptomatic and mild localised infections of the CNS, skin, eyes, or oral cavity can occur in 50% of infected infants (Nahmias *et al.*, 1971). The CNS involvement is very similar to that seen in disseminated infections, but the onset of illness is shorter and the mortality rate lower. Nevertheless, survivors usually experience residual brain damage (Whitley, 1984).

Skin infections, observed in 10% of infected newborns, are commonly acquired from HSV 2 or HSV 1 genital lesions of the mother (Feldman and Sonnabend, 1979). Recently however, van der Weil *et al.* (1985) have documented one case of paternal transmission from herpes labialis. An accepted approach to the prevention of neonatal HSV is to perform caesarian sections in pregnant women with known active or recent genital infection near term. Difficulties arise at the time of delivery when asymptomatic infections are encountered in

mothers with no known history of herpetic infection (Whitley *et al.*, 1980). Other difficult decisions have to be made when pregnant women, with a past history of infection or recent history of contact, seek reassurance that normal delivery will not expose the child to harm.

(vi) Infections of the Genital Tract

Genital herpes was first recorded by Astruc in 1736, an era when French prostitution was under medical surveillance (Hutfield, 1966). At this time no particular name was given to the condition, but Astruc recorded that it was a disease common to both men and women. This idea was refuted in the 19th century when Greenough suggested that the condition was unique to men, in particular those with a history of venereal disease. A few years later Unna reported the disease in 4.7-9.1% of prostitutes and stated that recurrences often coincided with menstruation (Hutfield, 1966).

In the 20th century genital herpes has become one of the most notorious sexually transmitted diseases. Changing attitudes towards sexual behaviour in the 1960s caused an upsurge in the number of genital HSV infections. In the 1980s, the incidence of the disease is still thought to be increasing, although the problem has been much exaggerated by the media. Scare-mongering allegations that HSV could be contracted from inanimate objects such as toilet seats, have now been dismissed by laboratory findings (Becker and Nahmias, 1985). However, many cases of anxiety and depression are still encountered in infected individuals, due to the social stigma of the recurrent disease.

Until the 1970s HSV 2 was thought to be the cause of all genital infections, with HSV 1 producing infections at sites above the waist. Then in 1971 and 1973 results of studies in the USA (Nahmias et al., 1971) and UK (Smith et al., 1973) indicated that between 3.4 and 16.9% of all individuals with genital herpes were infected with HSV 1. In 1977, Kalinyak et al. reported an extremely high incidence (37%) of genital HSV 1 in a population of college women, although current figures now suggest that 20-33% of all genital HSV infections will be caused by HSV 1 (McCaughtry et al., 1982; Docherty et al., 1984). (The implications of these figures are discussed in the next section - Orogenital Infections).

Rectal and perianal infections with either HSV 1 or HSV 2 are being increasingly recognised in the form of HSV proctitis. Although a large proportion of the cases are seen in homosexual men, the condition is also prevalent in heterosexual women who engage in ano-rectal intercourse (Corey and Spear, 1986b).

Epidemiological studies clearly indicate that a high proportion of HSV infections are being transmitted from asymptomatic sexual consorts (Mertz et al., 1985). Recent figures from the USA (Becker and Nahmias, 1985) suggest that as many as 75-90% of patients who demonstrated HSV 2 antibodies in their serum were unaware of having experienced any clinical manifestations of herpes. Thus, individuals with asymptomatic infections clearly present a high risk to any sexual partner (Rooney et al., 1986).

(a) Clinical manifestations

In males, multiple or grouped vesicles and ulcers may appear on the glans, prepuce or shaft of the penis, although herpetic urethritis and anorectal herpes are also common. In women, the main site of involvement is the cervix, which is usually affected at a subclinical level. Painful ulcerations of the vulva and vagina may also occur with accompanying features such as adenopathy (Feldman and Sonnabend, 1979).

Patients experiencing primary genital herpes frequently have systemic manifestations (eg generalised lymphadenopathy); however individuals with initial or recurrent genital infections tend to have a milder course of illness.

A history of itching, pain and/or sacral neuralgia 3-48 hours prior to the appearance of lesions, is particularly important in the diagnosis of recurrent disease. However, the frequency of recurrences appears to differ among individuals and is known to be influenced by the serotype of HSV with HSV 2 infections more prone to recurrences than HSV 1 infections (Corey *et al*., 1983).

(b) Prevention

At present, condoms are thought to provide an effective barrier to virus transfer, although it is recognised that the only absolute prevention is to avoid contact with lesions of an infected partner (Feldman and Sonnabend , 1979). Difficulties arise when asymptomatic infections occur and precautions are not taken, or when partners are unaware of the risks of oro-genital transfer and other mechanisms of direct or indirect transmission.

(c) Association with carcinogenesis

The increased prevalence of genital herpes, coupled with an increased incidence of cervical carcinoma in young women, prompted many epidemiological and ser ological investigations to establish an association between HSV and cervical cancer (Rawls et al., 1969; Barton et al., 1982; Nahmias et al., 1973). It is now recognised that cervical cancer and infection with HSV 2 occurs more frequently in women with multiple sex partners and who have first coitus at an early age (Stravasky et al., 1983). Examinations of patients with cervical neoplasia have demonstrated the presence of HSV proteins (Aurelian et al., 1981) and viral RNA in cancer cells and tissues (McDougall et al., 1980; Eglin et al., 1981). HSV DNA has also been reported in one cervical tumour (Frenkel et al., 1971), although subsequent studies have been unable to confirm this result (zur Hausen et al., 1974; Pagano, 1975). A limited portion of the HSV genome has, however, been demonstrated by in situ hybridisation of cervical carcinoma sections (McDougall et al., 1980; Eglin et al, 1981). In such cases the use of cloned, sub-genomic fragments of HSV 2 DNA allowed the authors to define specific regions (0.7-0.84 0.85-0.87, 0.88-0.98 map units) of the HSV 2 genome that were transcribed in 60% of cervical biopsies. In addition, McDougall et al. (1982) and Park et al. (1983), have detected a further region (0.58-0.63 map units), in 10-12.5% of cervical specimens, that may be associated with the transformation process.

At present a specific role for HSV in the process of carcinogenesis has not been determined. Two possible mechanisms for transformation have been suggested. One, the 'hit and run' theory,

proposes a fortuitous involvement of HSV in carcinogenesis (Minson et al., 1976; Skinner, 1976; Galloway and McDougall, 1983), whereas zur Hausen (1982) postulates that HSV may interact as a co-carcinogen with other agents (such as HPV), known to infect the cervix. To date, no firm evidence in support of either hypothesis is available (Cox et al., 1986; Grussendorf-Cohen et al., 1986).

(vii) Oro-genital Infections

Recent surveys of the sexual practices in homosexual and heterosexual populations report a high incidence of oro-genital contact in the sexually active 15-24 year old age groups (Chacko et al., 1982; Soendjojo, 1983). These findings have also been substantiated by the large number of HSV 1 genital infections found in American college students (male 26%, female 37%), with the most likely transmission being by oro-genital contact (McCaughtry et al., 1982; Docherty et al., 1984).

Despite the possibility that many oral HSV 2 infections may be contracted during oro-genital relationships, very few cases of oral HSV 2 isolations have been recorded (Kaufman and Rawls, 1972; Nahmias and Roizman, 1973; Wolonitis and Jeansson, 1977; Schlesinger et al., 1978; Tustin and Kaiser, 1979; Corey et al., 1983). Indeed the highest isolation rate was found in a select group of patients known to be engaged in oro-genital sex with people who had a part history of genital HSV 2 infections (Corey et al., 1983). In this case 11% of patient with primary genital herpes had HSV 2 isolated from the pharynx, however HSV isolations were also obtained from

patients suffering from non-primary initial (1%) or recurrent (1%) genital infections. Many of the patients had recently been diagnosed as having streptococcal pharyngitis since the accompanying symptoms included mild erythema, ulcerative pharyngitis, headaches, fever and malaise.

A more recent attempt to determine the incidence of oral HSV 2 infections has been carried out by Docherty _et al_. (1985). In their study of 69 students, 55 were found to have clinical symptoms of oral infection. However, only 43 HSV samples were isolated and all were typed as HSV 1. By interview it was determined that the severity of HSV infection bore no relationship to the sexual behaviour of the individuals concerned.

It has been suggested that the low recovery rate of oral HSV 2 may, in part, be due to the acquisition of HSV antibodies from a prior infection in the oral region (Docherty _et al_., 1985). Thus, exposure to oral HSV 2 would not necessarily produce a primary infection, and instead an asymptomatic or mild initial infection may occur. In other STDs (eg Neisseria gonorrhoea) isolation from apparently asymptomatic individuals is common (Weisner _et al_., 1973; Young and Bain, 1983).

(viii) Herpetic Infections in Renal Transplant Recipients

Viral infections associated with the herpesviruses are known to cause major complications for organ transplant recipients receiving immunosuppressive therapy (Pass _et al_., 1979; Dummer _et al_., 1985; Locksley _et al_., 1985; Weirtheim _et al_., 1985). In many

cases HSV infections are severe and result in prolonged viral shedding (Whitley, 1984) that usually occurs within 30 days of transplantation (Walker et al., 1982).

Serological data has confirmed that the majority of clinical manifestations of HSV following renal transplantation are due to reactivations of the latent virus (Warrell et al., 1980). However, it has been established, both for HSV and CMV infections, that the kidney itself can be a source of viral infection (Berglin et al., 1982; Weirtheim et al., 1983; Grundy et al., 1986).

(a) Clinical manifestations

In immunosuppressed individuals HSV infections may extend into the mucosal and deep cutaneous layers of the skin. Accompanying features such as necrosis, bleeding and pain, coupled with the inability to eat or drink, are frequently experienced (Naraqi et al., 1977; Pass et al., 1979; Meyers et al., 1980). In the first month following renal transplant, cold sores commonly occur but subsequent infections tend to be less common and usually return to the same frequency as that before transplantation.

(b) Immunosuppressive therapy

For many years azathioprine (a derivative of 6-mercaptopurine) has been the standard method of long-term immunosuppression in most transplant centres. The dose range of 2-5mg/kg is adjusted according to the white cell count and renal function.

Corticosteroids such as prednisolone are also used as a major immunosuppressive therapy because of their anti-inflammatory effect and stabilisation of the lysosome membranes (Chi sholm, 1983).

Despite previous debates about the optimum dosage of prednisolone, most units now follow the 'Belfast regime'; a low-level dose of steroids in the early post-transplant period (McGeown *et al*., 1977). Cyclosporin A (CsA), a fungal cyclic-peptide, is commonly used as an alternative to azothioprine. Although it has been shown to be an effective immunosuppressant several reports have expressed concern regarding the long-term effect of CsA therapy and oncogenesis (Sheil, 1984; Thompson *et al*., 1985). Current recommendations suggest that CsA treatment is safe when used only in patients who have immediate function of their kidney grafts after transplant (Chisholm, 1983).

GENOMIC ORGANISATION OF THE HERPESVIRIDAE

Classification of the Herpesviridae

The current classification of the members of the family Herpesviridae is based both on biological properties and sequence arrangement of the viral DNA (Table 1). This allows division of the viruses into three subgroups, the alpha herpesviridae (α), the beta herpesviridae (β) and the gamma herpesviridae (γ).

(a) Alpha herpesviridae (e.g. HSV, PRV)

Classification is based on a variable host range and short reproductive cycle. In cell culture they produce a rapid cytopathic effect and destruction of the infected cells. In animals, a latent viral infection is frequently established, found mainly in the ganglia.

Table 1 Characteristics of Selected Herpesviruses (Adapted from Roizman and Batterson, 1985)

Designation	Common Name	Subfamily	Genome Properties G + C (mole %)	Group Type*	MWT (x 10^6)
Human herpesviruses					
1	Herpes simplex virus type 1 (HSV 1)	α	67	E	96
2	Herpes simplex virus type 2 (HSV 2)	α	69	E	96
3	Varicella-zoster virus (VZV)	α	46	D	100
4	Epstein-Barr virus (EBV)	γ	59	C	114
5	Cytomegalovirus (CMV)	β	57	E	145
Mammalian herpesviruses					
Bovine herpesvirus 1	Infectious bovine rhinotracheitis virus (IBRV)	α	72	D	-
2	Bovine mammillitis virus (BMV)	α	64	E	88
Equine herpesvirus 1	Equine abortion virus (EAV)	α	57	D	94
Suid herpesvirus 1	Pseudorabies virus (PRV) Aujeszky's disease virus	α	74	D	91
Ateline herpesvirus 2	Herpesvirus ateles (strain 810)	γ	48	B	90
Saimirine herpesvirus 2	Herpesvirus samiri (HVS)	γ	46	B	103
Non-mammalian herpesviruses					
Ictalurid herpesvirus 1	Channel catfish virus (CCV)	α	56	A	86

* Genome arrangements are represented in Fig 3.

(b) Beta herpesviridae (e.g. CMV)

The members of this subgroup have a relatively narrow host range and slow reproductive cycle. *In vitro*, the infection progresses slowly, with cell enlargement being a frequent occurrence, although a similar enlargement of the infected cells may also be found *in vivo*. The viruses can be maintained in a latent form in the kidneys, secretory glands, lymphoreticular cells and other tissues.

(c) Gamma herpesviridae (e.g. EBV, HVS)

A characteristic of the members of this subgroup is the extremely narrow host range, being limited to the family, or order, of the natural host. In cell culture, replication occurs mainly in lymphoblastoid cells.

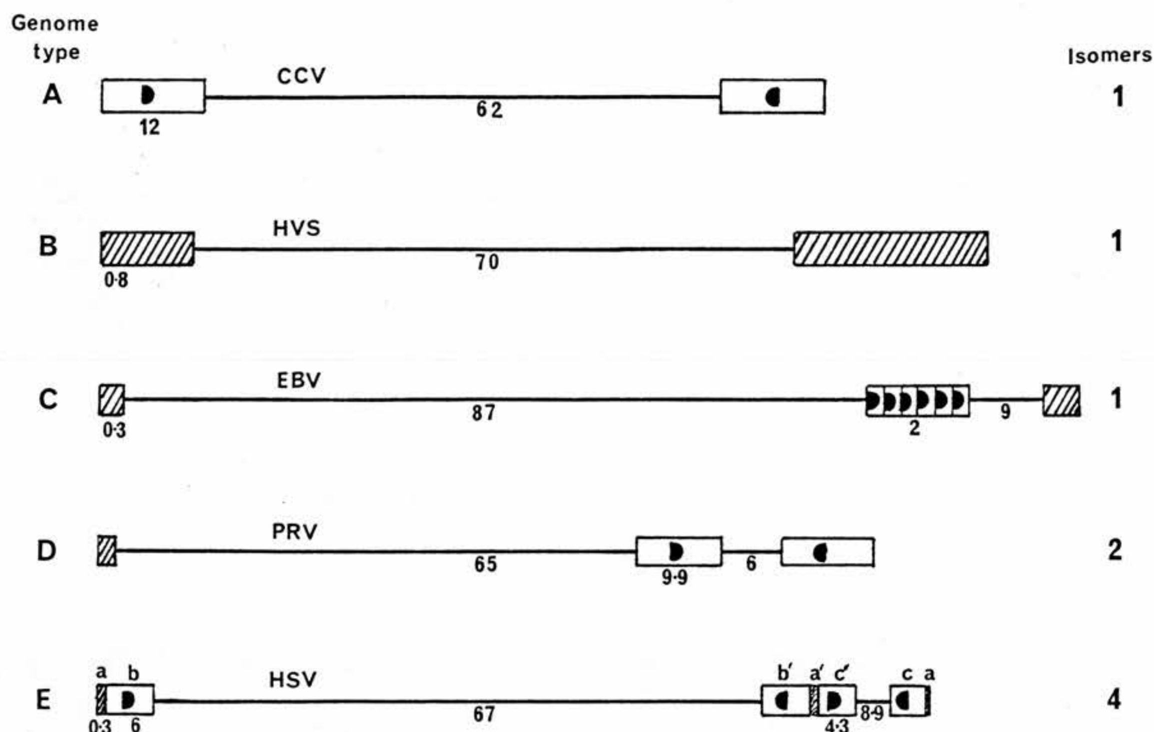
Genomic Arrangements of the Herpesviridae

The DNAs of the herpes viruses differ in size, base composition and structural organisation; however, all members of the herpes family can be represented by one of five arrangements, as shown in Fig 3.

The genomic structures of the five human herpes viruses (VZV, CMV, EBV, HSV 1 and HSV 2) are complex arrangements of unique DNA sequences and repeated regions. In HSV and CMV, the genomes (Type E) are found in four isomeric forms; however, the DNA molecules of VZV possess only one set of inverted repeats, bracketing the S segment (Type D). In this case inversion of the S segment produces only two isomeric forms. The DNA of EBV is completely different

Figure 3

A schematic diagram of the five types of genomes found in the family Herpesviridae (adapted from Roizman and Batterson, 1985).



The horizontal lines represent unique sequences and the numbers below represent millions of molecular weight.

◗ - reiterated sequences (excluding terminal reiterations). The direction indicates the orientation (◗ direct, ◖ indirect) of the sequence.

Where more than one set of large repeats has been reported they are designated as lower case letters, e.g. b and c reiterated sequences in HSV DNA).

//// - terminal sequences. (All terminal reiterations are direct repeats). Numbers below refer to the molecular weight of a unit length of the genome.

from the other human herpes viruses and is categorised in genome type C, which are found in only one isomeric form.

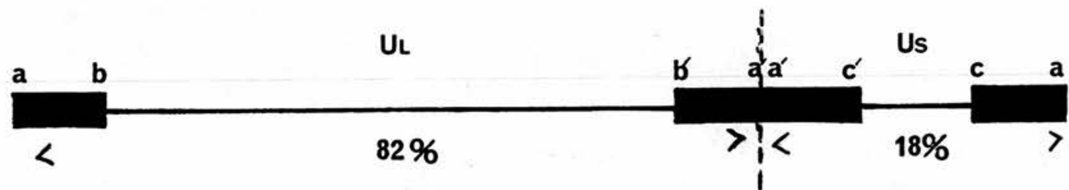
The DNAs of equine abortion virus (EAV) and pseudorabies virus (PRV) possess a similar genomic structure to that of VZV (Genome type D), despite the fact they originate in non-primate mammals. Another mammalian herpes virus bovine mamillitis virus (BMV) has a DNA structure very similar to that of HSV (Genome type E), particularly in the reiterated sequences of the terminal regions (Buchman _et al_., 1979). Herpesvirus samiri (HSV) and herpesvirus ateles (HVA) DNA have substantially different genomic structures from the other herpesviruses (Genome type B). Although the channel catfish virus (CCV) shares a similar structure, having only one isomeric form, differences in the size and G + C base composition of the genome are apparent (Genome type A). HVS and HVA genomes are much larger consisting of a lengthy unique sequence, bracketed by a number of tandemly reiterated G + C rich sequences.

Structural Organisation of the HSV Genome

The genomes of HSV 1 and HSV 2 are linear, double-stranded molecules of DNA (96×10^6 molecular weight (mwt)), having a base composition of 67 and 69% G + C respectively. The DNA structure (Fig 4) consists of two covalently linked components, designated the long (L) and the short (S) segments, that are composed primarily of unique sequences (U_L and U_S). The long segment is bracketed by 9Kb inverted repeats (_a_ _b_ and _b'a'_) and the short segment by 6Kb inverted repeats (_a'c'_ and _c_ _a_), that differ in base composition

Figure 4

The genome arrangement of HSV DNA.



U_L - long unique region

U_S - short unique region

ab, b'a' - U_L reiterated sequences

ac, c'a' - U_S reiterated sequences

from the inverted repeats of the long segment (Hayward *et al.*, 1975a and b; Wadsworth *et al.*, 1975). A number of tandemly repeated sequences (designated as the *a* sequence) are located at the termini of the long and short segments (Grafstrom *et al.*, 1975; Wadsworth *et al.*, 1975). Thus, the 'idealised' sequence arrangement for HSV DNA is $\underline{a}_L \underline{a}_m \underline{b}$ - U_L - $\underline{b}'\underline{a}'_n\underline{c}'$ - U_S - $\underline{c}\ \underline{a}_S$, where the underlined letters represent inverted repeats, and the subscripts m is $0 > 10$, and n is $1 > 10$ (Roizman and Batterson, 1985). Alterations in the number or size of the *a* sequence may result in inter- or intra-strain variability (Roizman and Tognon, 1983; Hayward *et al.*, 1984; Roizman and Batterson, 1985).

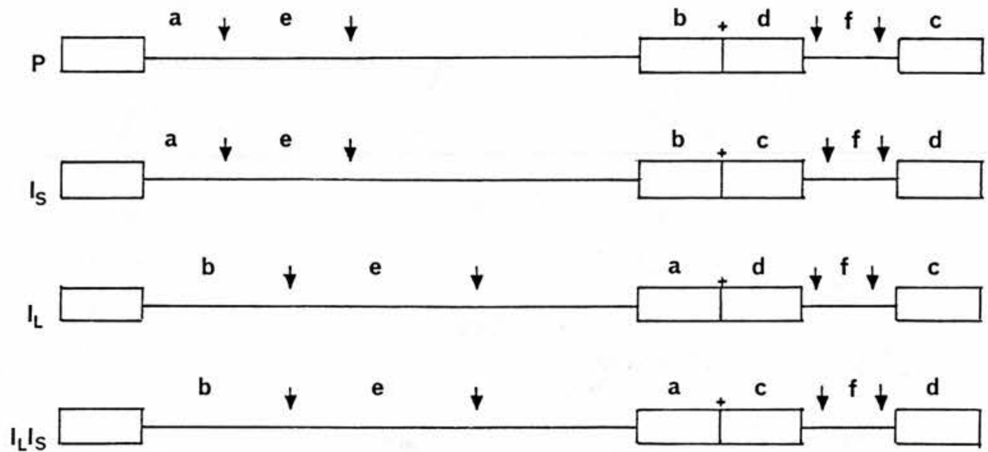
(a) Isomeric Arrangements of HSV DNA

In 1975 Sheldrick and Berthelot suggested that the L and S components could invert relative to each other. Subsequently it was proven that DNA extracted from wild-type virions actually contained an equimolar mixture of four isomeric forms, produced by the combination of backward or forward orientations of the L and S segments (Fig 5) (Hayward *et al.*, 1975a; Wadsworth *et al.*, 1975; Delius and Clements, 1976).

The four isomeric forms were designated as; prototype (P); inversion of the S component (I_S); inversion of the L component (I_L); and inversion of both S and L components (I_{SL}) (Roizman *et al.*, 1974; Hayward *et al.*, 1975a and b). As a consequence of the L and S inversions, cleavage by restriction enzymes, that cut only in the unique sequences (eg *EcoRI*), gives rise to three sets of fragments (Fig 5).

Figure 5

The isomeric arrangements of HSV DNA.



↓ = cleavage site

Fragments from the termini are generated once in two isomeric forms (e.g. a from the Long segment is generated once in P and once in I_S) therefore are present 1/2 = 0.5M amounts relative to the intact DNA).

Fragments obtained from the joint segments (e.g. b + d, or a + d) are generated once in four isomeric forms, therefore are present 1/4 = 0.25M amounts relative to the intact DNA.

Fragments from the Unique sequences occur in all four isomers, thus are present in 4/4 = 1M amounts relative to the intact DNA (e.g. e or f).

(i) Four terminal fragments (a,c,b,d) each present in 0.5M concentrations, relative to the molarity of the intact DNA.

(ii) Four fragments from the 'joint' region, spanning the L and S components (b+d, b+c, a+d, a+c), each present in 0.25M concentrations, relative to the molarity of the intact DNA.

(iii) A number of fragments situated between the terminal and junction fragments; each present in 1M concentrations relative to the intact DNA (Hayward et al., 1975a).

Although the number of fragments varies depending on RE cleavage sites, the profiles obtained by electrophoresis on agarose gels consists of a complex mixture of molar, half molar and quarter molar fragments (Fig 6). Each fragment differs accordingly in intensity when the gel is stained with ethidium bromide. Exceptions occur when multicut restriction enzymes are used. For example, the restriction enzyme HpaI cuts both in the unique sequences and the inverted repeats as shown in Fig 7. Since the four genome arrangements are present in equal amounts the cleavage produces:

(i) Five, 1 molar fragments (i, e, g, h, f) generated from the unique regions and the end repeats cut by HpaI.

(ii) Four, 0.5M fragments two of which (c and d) are generated from the ends containing the uncut repeats. The remaining two (h+c and h+d) are generated from areas between the cleavage sites in the internal repeats and the unique sequence in U_S. The short unique region has

Figure 6

Submolar fragments displayed in the restriction enzyme cleavage patterns of HSV DNA electrophoresed on agarose gels.

BglII / HSV1

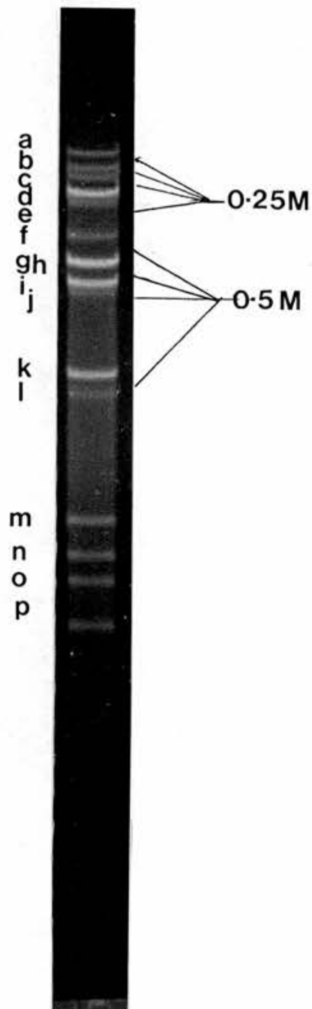
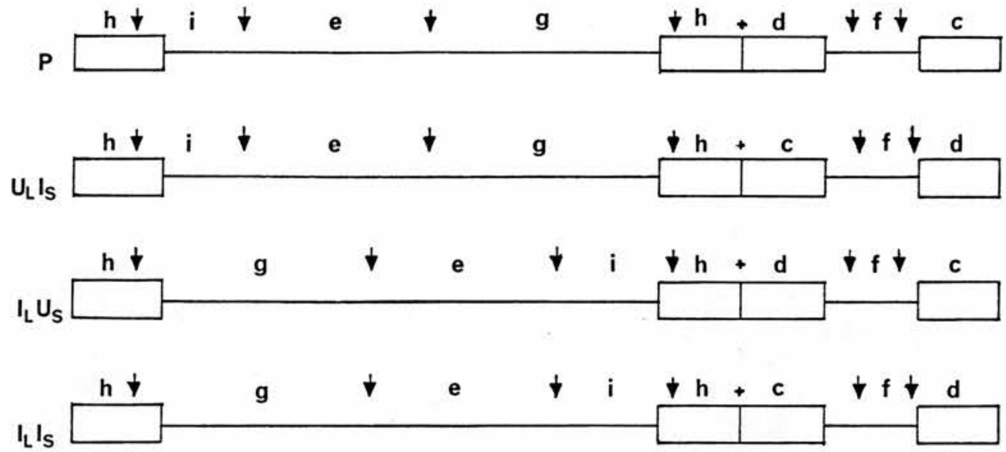


Figure 7

Schematic representation of the molar ratios of fragments in HSV 1 produced by cleavage with HpaI.



↓ restriction enzyme cleavage site.

1M fragments: i, e, g, h, f

0.5M fragments: c, d, h + d, h + c

No 0.25M fragments.

no cleavage sites in its flanking repeats. Thus, inversions of the unique region containing the cut flanking repeats, have no effect on the molar ratios of the fragments produced in the long region. Since a similar effect is produced by the fragments flanking the short unique region, no 0.25M fragments are generated.

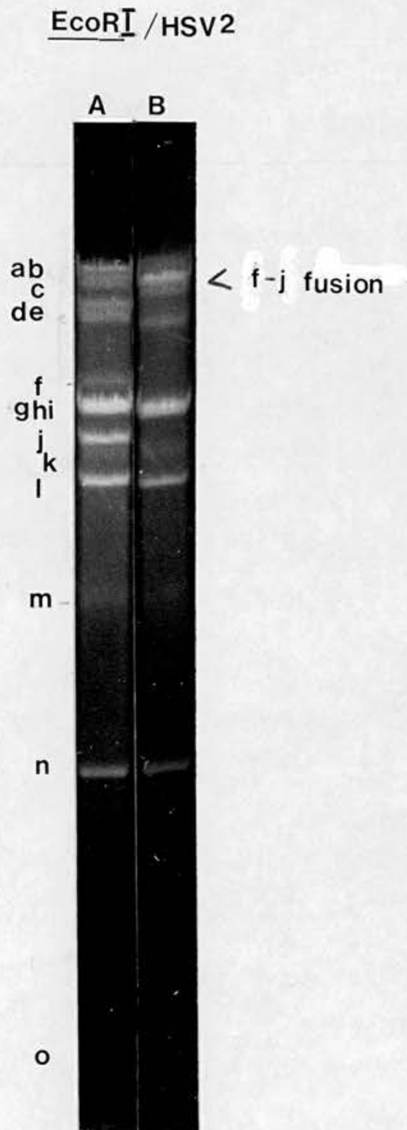
(b) Variability of the Restriction Endonuclease Profiles of HSV DNA

Comparison of the RE profiles of HSV DNA with the relevant standard strain(ie HSV 1 (17syn$^+$) or HSV 2 (HG52)) reveals two classes of variation: (i) presence or absence of RE cleavage sites, and (ii) mobility variations mainly due to variations in the number of tandemly repeated sequences.

The first type of variability gives rise to a change in fragment number and molecular weight. Presence of an additional RE cleavage sites results in the disappearance of a fragment and the appearance of two new fragments. The sum of the molecular weights of both new fragments is equivalent to that of the missing fragment. Absence of a particular RE cleavage site results in the disappearance of two (or more) adjacent fragments and the appearance of a larger fusion fragment. The combined molecular weights of the missing bands, which are always contiguous in the physical maps, are equivalent to the molecular weight of the fusion fragment (Fig 8). The second type of variability results in the alteration of fragment molecular weight by more than ten per cent (Buchman *et al*., 1980). This is mainly due to reduction or

Figure 8

Major variations in the restriction enzyme profiles of HSV 2 DNA.
Tracks A, prototype
B, f-j fusion



The 'spliced' gel photograph in this figure has been taken from a single agarose gel. This condition also applies to all other 'spliced' photographs in subsequent figures.

amplification of the a sequences (Hayward et al., 1975a and b; Wagner and Summers, 1978; Locker and Frenkel, 1979). (See Results section)

It is well documented that alterations in the a sequences results in inter- and intra-strain mobility variations (Hayward et al., 1975b; Wagner and Summers, 1978; Locker and Frenkel, 1979; Roizman and Tognon, 1982; Roizman and Batterson, 1985). However, variability in the electrophoretic migration of non-terminal fragments has also been recorded. Locker and Frenkel (1979) described minor size heterogeneities in the U_L segment of three USA standard strains (HSV 1 (F), (Justin) and (VR-3)) when digested with BamHI, KpnI and SalI enzymes. Analysis of plaque-purified HSV 1 stocks has also revealed non-terminal fragment variations (Roizman and Tognon, 1982). In this case although the variable fragments mapped near the termini, the variability was thought to be unrelated to the a sequences. Further plaque purification of variants showing rapidly or slowly migrating fragments allowed the subsequent analysis of individual plaque-picked virus stock. In each stock, alterations in fragment mobility were observed to vary from fast to slow migration and vice versa.

Variations in the unique sequences of clinical isolates (both HSV 1 and HSV 2) have also been documented by Chaney et al. (1983a and b). The HSV 1 genome showed variations in both the short (e.g. Bamz) and the long (eg EcoR1) unique regions. By comparison the HSV 2 genome exhibited only two regions of variability (Bamy and Bgli) both in the long unique segment. Variations in the short unique region were observed in subterminal fragments.

A Comparison of HSV 1 and HSV 2 Genomes

The structure and sequence arrangements of the HSV 1 and HSV 2 genomes are very closely related (Kieff *et al*., 1971; Kieff *et al*., 1972) having almost complete equivalence in the location of homologous sequences and functions throughout the L region. In addition the positions of the U_S/U_L junctions are reported to be tightly conserved between the two genomes (Hayward *et al*., 1984). However, detailed differences have been observed and are as follows:-

(i) The S unique region exhibits a three kilobase size increase in HSV 2 compared with HSV 1 (Hayward *et al* 1984).

(ii) The nucleotide sequences of the terminally redundant *a* sequences have been shown to differ between HSV 1 and HSV 2 (Davison and Wilkie, 1981).

(iii) The glycoprotein C region is known to encode a 130K glycoprotein in HSV 1, but a serologically poor cross-reacting 75K glycoprotein in HSV 2.

(iv) Initially glycoprotein gG was thought to be found in HSV 2 and not HSV 1, however Richman *et al*. (1986) have recently reported a corresponding gG in HSV 1.

(v) Approximately 30% of the nucleotide sequences of the thymidine kinease genes differ for HSV 1 and HSV 2 (Hayward *et al*., 1984).

Inter-relationships of the Herpesviridae

Molecular hybridisation experiments have provided additional evidence of an association between particular herpesviruses. The DNA from some members of the alphaviridae (HSV, BMV, EAV, PRV and VZV) have been found to have equivalence in the L segment near the origin of replication and the region specifying the DNA-binding protein (Hayward *et al*., 1984). The HSV 1 and HSV 2 genomes share approximately 50% of their sequences (Kieff *et al*., 1972); the regions of least homology occurring mainly in the S segment and the inverted repeats of the L segment (TR_L/IR_L) (Wilkie *et al*., 1979). PRV DNA has shown 8-10% homology with HSV 1 and HSV 2 DNA (Davison and Wilkie, 1983) whereas EAV and VZV DNA is known to contain homologous sequences to those of the HSV (1 and 2) genomes.

THE APPLICATION OF RESTRICTION ENDONUCLEASE ANALYSIS IN EPIDEMIOLOGICAL STUDIES

The introduction of polypeptide analysis in 1974 (Heine *et al*., 1974) demonstrated the usefulness of molecular technology in the investigation of intratypic variations. Two years later Pereira *et al*. (1976) used a similar technique to examine the variations in 53 HSV 1 isolates. In this instance heterogeneity in the electrophoretic mobilities of seven structural polypeptides permitted subdivision of the isolates into specific groups. Indeed the identification of paired isolates by this method provided the first molecular tool for epidemiological tracing. Parallel studies (Hayward *et al*., 1975a) on the nucleotide sequence of HSV DNA

introduced a superior technique for identifying isolate uniqueness (Lonsdale *et al.*, 1979). Since that time restriction endonuclease studies have provided invaluable information about the epidemiology and genetic variability of HSV. The following sections provide a brief account of the applications of RE analysis in epidemiological studies and are summarised in Table 2.

(a) Transmission of HSV

In several studies (Buchman *et al.*, 1978; Linneman *et al.*, 1978; Halperin *et al.*, 1980) the variability in RE profiles has been used to trace the spread of virus from one individual to another. Much of the early work involved the study of HSV 1 infections in paediatric intensive care units (PICU). Buchman *et al.* (1978) investigated an outbreak of HSV infection in patients, nurses, one spouse and a dental pathologist. By analysis of the RE profiles and the clinical information it was established that a single HSV 1 strain was not responsible for all the infections in the outbreak. Instead, two independent introductions of HSV 1 had occurred; one from a patient and the other from an asymptomatic nurse.

In the same year, Linneman *et al.* (1978) used RE analysis to examine HSV 1 isolated from two infants in a newborn nursery. No differentiation could be made between the isolates in this instance therefore it was suggested that person-to-person transmission had occurred.

Subsequent studies using RE analysis to trace nosocomial transmission have followed a very similar pattern, either proving or disproving transmission in a small number of individuals (Buchman *et*

al., 1979; Halperin et al., 1980; Buchman et al., 1980; Hammerburg et al., 1983; Sakaoka et al., 1984).

Evidence of adult transmission has also been investigated by RE analysis. In 1985 van der Weil et al. illustrated the need for caution when a family member suffers herpes labialis. In this instance a disseminated HSV 1 infection in a newborn infant was acquired from the mother who had been infected by her husband during a recurrence of herpes labialis. Caution is also required by dental personnel prone to herpetic whitlows. Manzella et al. (1984) have reported the transmission of HSV 1 stomatitis from dental hygienists to patients.

More recently Davis et al. (1985) and Rooney et al. (1986) have examined the transmission of HSV 2 between sexual partners. Davis et al. used RE analysis to confirm that an identical HSV 2 strain was isolated from supposed sexual partners, and Rooney et al. demonstrated the transmission of HSV 2 from an asymptomatic sexual partner.

(b) Evidence of concurrent or concommitant HSV infections

Concomitant and concurrent HSV infections have also been investigated by RE analysis. Embil et al. (1981) showed that concurrent penile and oral lesions from a single patient had been caused by the same HSV 1 strain, whereas Fife et al. (1983) reported two cases of concomitant genital infection with both HSV 1 and HSV 2. The RE data and clinical information led the authors to suggest that either a concurrent, or successive infection with HSV 1 and HSV 2 had occurred. Subsequently both serotypes had become latent in

one or more ganglia, and on reactivation had caused concomitant genital infection. Similarly Whitley et al. (1982) studied concomitant brain and oro-labial HSV isolates from eight patients who had HSV encephalitis. Paired isolates from five patients were found to be identical, with one of the five having had a primary infection. This was therefore direct evidence that HSV encephalitis could follow either a primary, or recurrent infection. The discovery of non-identical HSV isolates in three patients demonstrated that patients could be infected by two different virus strains in the brain and oro-labial sites. Subsequent studies by Dix et al. (1983) and McFarlane et al. (1985) have confirmed the isolation of concomitant HSV 1 from brain and oro-labial sites, where Gerdes et al. (1981) have reported a case of concurrent HSV 1 infections in two brain sites.

(c) Evidence of reinfection with HSV

Restriction endonuclease analysis of genital HSV 2 infections (Buchman et al., 1979) has provided unambiguous evidence of multiple infections in patients with recurrent genital lesions. Serial isolations of non-identical HSV 2 strains from one of the eight patients examined, led the authors to suggest that individuals previously infected with HSV 2 could be re-infected at the same or nearby site.

A similar account of reinfection with HSV 2 has also been provided by Maitland et al. (1982). In this study a non-identical HSV 2 strain was recovered from one of thirty patients with recurrent genital herpes. The frequency of reinfection with new HSV

strains was investigated by Schmidt _et al_. (1984) who studied 45 patients with symptomatic recurrent genital herpes. However, in this case all symptomatic recurrences were found to be caused by reactivation of the latent virus and not by reinfection with a new HSV strain.

A novel account of reinfection was documented in 1983 by Kit _et al_. During the first symptomatic episode of genital herpes, HSV 1 was isolated from the lesions of a single patient. However, subsequent isolations from three successive episodes (recurring four, seven and nine months after the HSV 1 infection) were found to be caused by identical strains of HSV 2. Serological analyses suggested that the patient had been exposed to HSV 2 prior to the HSV 1 genital infection, therefore the authors concluded that HSV 2 infections are more likely to recur than their HSV 1 counterparts.

Reinfection and concurrent infection in the ganglion has also been investigated by RE analysis. Lewis _et al_. (1984) reported the recovery of non-identical strains of latent HSV 1 within the trigeminal nerve complex of two individuals. In this instance, no distinction could be made between exogenous reinfection or concurrent infection with more than one strain of HSV because clinical information was lacking.

(d) Strain variations within the population

The majority of epidemiological studies examining the RE variations of HSV within a population have concentrated on small numbers of patients (Table 2), although multiple HSV isolates from individual patients have been examined. A comparative analysis of

Table 2

A summary of the applications of REA (HSV 1 isolates unless otherwise stated)

Authors			No of patients examined
(a) Transmission of HSV			
(i) Neonatal			
Buchman *et al.*	1978		12
Linneman *et al.*	"		4
Buchman *et al.*	1979		8
Halpern *et al.*	1980		4
Buchman *et al.*	"		2
Adams *et al.*	1981		4
Hammerburg *et al.*	1983		4
van der Weil *et al.*	1985		3
Sakaoka *et al.*	1984		6
(ii) Stomatitis			
Manzella *et al.*	1984		6
(iii) Sexual			
Mertz *et al.*	1985		20
Davis *et al.*	"		6
Rooney *et al.*	1986		3
(b) Concomitant/concurrent HSV infection			
Embil *et al.*	1981		1
Gerdes *et al.*	"		
Whitley *et al.*	1982		1
Fife *et al.*	1983		2
Dix *et al.*	"		1
McFarlane *et al.*	1985		2
(c) Reinfection with HSV			
Buchman *et al.*	1979	(HSV 2)	8
Maitland *et al.*	1982	(HSV 2)	30
Kit *et al.*	1983		1
Lewis *et al.*	1984		18

(d)/..

Table 2 (continued)

Authors			No of patients examined
(d) Strain variability of HSV			
Buchman *et al.*	1978	(HSV 2)	8
Lonsdale *et al.*	1979		17
Brown	1980		18
Maitland *et al.*	1982	(HSV 2)	30
Sheppard *et al.*	"	(HSV 2)	9
Barton *et al.*	"	(HSV 1 + 2)	25
Chaney *et al.*	1983a	(HSV 1)	29
		(HSV 2)	38
Chaney *et al.*	1983b		84
Sakaoka *et al.*	1985		93
Sakaoka *et al.*	1986		125

genital HSV 2 isolates from 38 patients was carried out in 1983 by Chaney _et al_. (a). In this instance the isolates were collected from two geographically distinct areas in Canada. Also included in the study were 12 genital and 17 facial HSV 1 isolates from each of the two locations in Canada. On the basis of RE analysis, no significant correlation between site of isolation and type of RE variation could be detected. However, major RE variations could distinguish all the HSV 1 isolates from each other; the HSV 2 isolates showed very little evidence of strain variability. A subsequent paper by Chaney _et al_. (1983b) extended the study of HSV 1 isolates to include 55 ganglion, brain and facial isolates obtained from the USA and Japan. Comparison of these isolates with the HSV 1 isolates from Canada, revealed a significant difference in the distribution of RE cleavage sites from each geographical location and different anatomical site.

The variations in HSV 1 strains from three different geographical locations in Japan have recently been investigated by Sakaoka _et al_. (1985). In this instance no significant differences in the distribution of RE variations were noted in isolates from patients living in close proximity (the areas of Torrori and Kawanga). Yet isolates from Sapporo, a geographically distinct area, showed a significant difference in the proportion of HSV 1 subtypes. Subsequent studies (Sakaoka _et al_., 1986) compared these results with 32 HSV 1 isolates from Kenya, and again the distribution of RE cleavage sites was found to differ significantly between the two countries.

Only one large scale study of HSV 2 variation has been attempted from 30 patients attending a single clinical centre (Maitland *et al.*, 1982). In this investigation 65 multiple genital HSV 2 isolates were examined by RE analysis, allowing the examination of *in vivo* variation within an individual. Using multicut enzymes (*BamHI* and *SstI*) considerable variability was observed between paired isolates from the same individual. Fragment variations observed for *BamHI* were assigned to the terminal or subterminal regions but no assignation could be made for the *SstI* results as no endonuclease map was available for consultation. Nevertheless, the results indicated that specific variation could be induced in the individual, despite the highly conserved nature of the HSV 2 genome.

Epidemiological Tracing of Herpesviruses Other Than HSV

The variability of RE cleavage sites has also been used for epidemiological tracing of other herpes virus infections; the following sections provide an outline but are not an extensive account for each virus.

(a) Human herpesvirus

Huang *et al.* (1980) have provided a detailed analysis of both *in vitro* and *in vivo* variations in CMV, a virus with a similar genomic arrangement to that of HSV. Analysis of plaque-purified standard CMV strains (AD169 and Towne) demonstrated that modifications of minor fragments could occur. Variations *in vivo* were analysed by the examination of four case studies, as outlined

in Table 3. In each case RE analysis demonstrated that in vivo rearrangements may have occurred which resulted in minor differences in the RE cleavage sites.

Additional epidemiological information with respect to the horizontal transmission of CMV has also been provided by neontal transmission (Huang et al., 1980; Spector, 1983; Hutto and Pass, 1984; Adler et al., 1985), neonatal-adult transmission (Spector and Spector, 1982; Dworsky et al., 1984) and sexual transmission (Hansfield et al., 1985). The transmission of CMV from donor organs to the recipient has also been investigated by RE analysis (Weirtheim et al., 1983; Grundy et al., 1986). Similar genomic differences have been used for epidemiological tracing in both VZV (Pinchini et al., 1983; Strauss et al., 1983; Embil et al., 1986) and EBV (Dambaugh et al., 1980) isolates.

(b) Non-human herpesvirus

RE analysis has also provided a useful tool for the differentiation and classification of non-human herpesviruses. Osorio et al. (1985) used a combination of RE analysis and serological data to define five major types of bovine herpes virus from the USA; infectious bovine rhinotracheitis virus (BHV 1), bovine herpes mammillitis virus (BHV 2), malignant catarrhal fever virus (MCF), bovine CMV-like virus and Pennsylvania 47 group virus. In the same way Sabine et al. (1981) and Studdert et al. (1981) have classified two groups of equine herpes viruses (EHV1) with respect to their RE cleavage profiles. More extensive RE analysis of foetal and respiratory EHV1 isolates (Allen et al., 1983a) confirmed the existence of two EHV subtypes and the apparent lack of heterogeneity

Table 3

Examples of the applications of RE analysis in the study of CMV infections

Subject	Author	
(i) Recurrent maternal infections	Huang *et al.*	1980
(ii) Transmission in consecutive congenital siblings	"	
(iii) Donor-recipient transmission following blood transfusion	"	
(iv) Persistent CMV infections	"	
Transmission from neonate to adults	Spector and Spector	1982
	Dworsky *et al.*	1984
Neonatal Transmission	Spector	1983
	Hutto and Pass	1984
	Adler *et al.*	1985
Sexual Transmission	Handsfield *et al.*	1986
Transmission of CMV from donor kidneys	Weirtheim *et al.*	1983
	Grundy *et al.*	1986

among EHV 1 isolates. In a subsequent study (Allen *et al*., 1983b) the authors also examined the alterations of EHV 1 isolates after serial passage in non-equine cell lines. The alterations, as determined by multicut enzymes, were thought to be due to sequence additions or deletions resulting in electrophoretic mobility variations of both terminal and unique fragments. Comparable work on the stability (Mengling *et al*., 1983; Wathen and Pirtle, 1984) and epidemiological differentiation (Paul *et al*., 1982; Pirtle *et al*., 1984) of PRV strains has also been made possible by means of RE analysis.

THE DEVELOPMENT OF NON-RADIOLABELLED PROBES FOR THE DETECTION OF NUCLEIC ACID SEQUENCES

In recent years DNA hybridisation probes have provided an invaluable tool for use in the investigation of a wide variety of conditions, including infectious disease, cancer, and genetic abnormalities. Originally the probes were prepared by labelling a specific DNA sequence with a radioisotope (such as ^{32}P), which could then be hybridised to target DNA and detected by autoradiography. However, limitations associated with the use of radioisotopes in routine diagnostic procedures have prompted the development of non-radiolabelled DNA detection systems.

The potential use of non-radiolabelled nucleic acid probes was first realised by Miller *et al* (1966) who observed that the carcinogen N-acetoxy-2-acetyl-aminofluorene (AFF) could bind to low levels of nucleic acids in rat liver. Some AFF was metabolised by

the rat to N-hydroxy-AFF which could be bound *in vitro* to high concentrations of nucleic acids by modification of the guanosine residues.

These modifications have more recently been further adapted to provide immunodetectable probes for *in situ* and blot hybridisations (Landegent *et al.*, 1984; Tchen *et al.*, 1984). Tchen *et al.* (1984) modified the nucleic acids (Fig 9) by means of an *in vitro* enzymic reaction with N-acetoxy-N-2AAF and its 7-iododerivative (AAIF) to make the nucleic acid immunogenic. The immunogenic nucleic acid probe could then be hybridised to immobilised target DNA and detected by an AAF-modified guanosine antibody and fluorescent-labelled second antibody. Similarly, Landegent *et al.* (1984) used comparable AAF-nucleic acid probes for the examination of microscopic preparations of mouse satellite and human CMV DNA. Both methods allowed single and double-stranded nucleic acids to be labelled and could easily detect complementary nucleic acid sequences. However, the use of AAF, a known carcinogen, made the procedure unsuitable for routine diagnostic application.

Many alternative methods employed for the production of non-radiolabelled probes involve labelling the nucleic acids with the vitamin, biotin (see Fig 10). In 1975 Manning *et al.* developed a method of electrostatically cross-linking biotin-labelled cytochrome C (a positively charged protein) to nucleic acid by treatment with formaldehyde but this procedure proved to be unsuitable since the probes were unstable under hybridisation conditions. Renz (1983) overcame this problem by covalently cross-linking biotin-labelled

Figure 9

Preparation and detection of immunonucleic probes (adapted from Tchen et al., 1984).

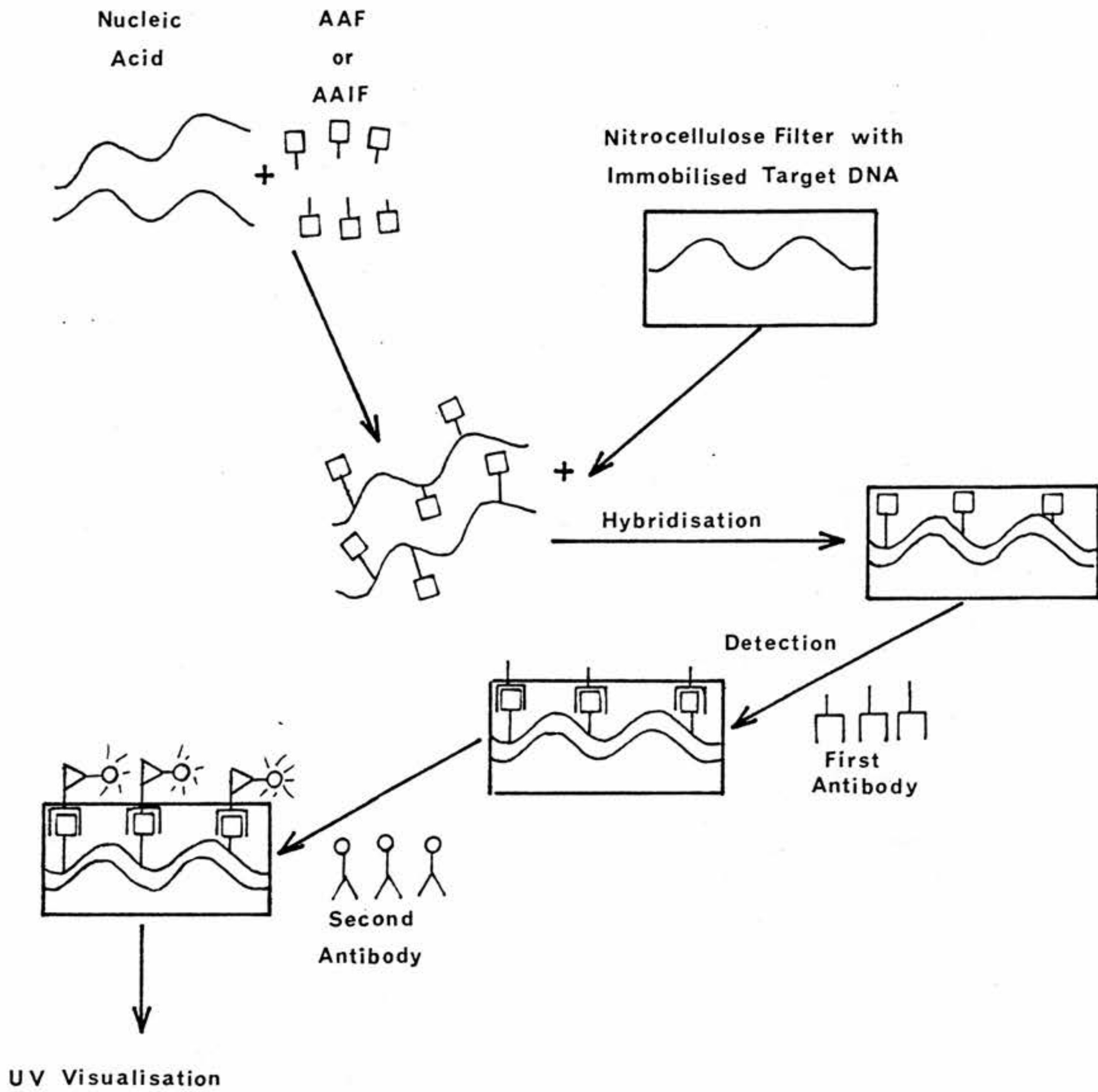
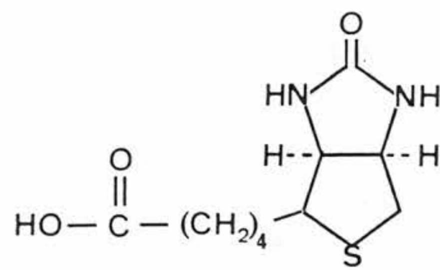


Figure 10

The structure of Biotin.



histone H 1 (a lysine-rich DNA-binding protein) to single-stranded nucleic acids in the presence of glutaraldehyde. After hybridisation, the target DNA was detected with an avidin-peroxidase conjugate; however, the detection appeared to be much less sensitive than procedures that exploited radioisotopes. Renz and Kurtz (1984) then avoided the use of avidin/biotin systems by cross-linking either peroxidase or alkaline phosphatase to a small (1.5Kb) synthetic polymer, polyethyleneimine. This method of electrostatic cross-linkage allowed simultaneous hybridisation with two DNA probes by use of two colorimetrically detectable enzymes (peroxidase and alkaline phosphatase) as the protein moiety of the probe. Complementary DNA sequences for each probe were visualised by development in two separate substrate solutions, thus allowing a colorimetric differentiation specific for the different probes.

An alternative method of preparing non-radiolabelled probes was developed by Langer _et al_. (1981), who incorporated biotin into DNA by the process of nick translation using biotinylated derivatives of uridine (UTP) and deoxyuridine triphosphate (dUTP). In this instance hybridisation signals could be detected by the appropriate immunofluorescent (eg antibody) or affinity (eg avidin) reagents. Two years later, Leary _et al_. (1983) reported an enzymic detection protocol for use in the visualisation of biotinylated UTP DNA probes (Fig 11). After nick translation and hybridisation the residual probe is removed by washing procedures before the nitrocellulose filters (containing immobilised nucleic acid) are incubated with an avidin or streptavidin (SA) complex and biotinylated polymers of intestinal alkaline phosphatase (B-polyAP). The nucleic acid

Figure 11

Visualisation of target nucleic acid sequences as described by Leary *et al*., 1983.

Nitrocellulose

Double-stranded DNA/RNA

Single-stranded immobilised target DNA/RNA

Hybridisation with Biotinylated Probe

B

B

B

B

Streptavidin (SA)

Incubation

B-poly (AP)

B-SA-B-poly (AP)

B-SA-B-poly (AP)

NBT and BCIP Dyes

Colorimetric Detection

sequences can then be visualised by incubation with a mixture of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT) dyes, resulting in a blue/purple colour development at the sites of hybridisation.

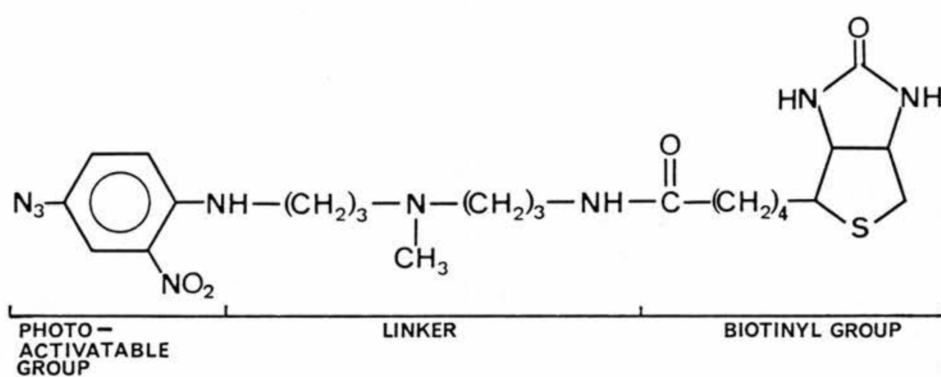
Recently Forster _et al_. (1985) have developed a novel method for labelling DNA with a photo-activable analogue of biotin; N-(4-azido-2-nitrophenyl)-N'-(N-d-biotinyl-3-aminopropyl)-N'methyl-1,3-propanediamine), more commonly called photobiotin (Fig 12).

Brief irradiation of photobiotin with visible light forms stable linkages with single or double-stranded nucleic acids, yielding a nucleic acid probe. Following normal hybridisation conditions target DNA can be detected by colorimetric development with biotinylated avidin-alkaline phosphatase conjugates as outlined by Leary _et al_. (1983).

A number of 'ready-to-use' kits are currently available for the detection and identification of specific nucleic acid sequences. These commercially prepared products include reagents for biotinylating DNA, detection kits, _in situ_ hybridisation kits and in some cases, specific biotinylated probes (eg HSV, EBV, chlamydia). Reports from the manufacturers indicate that non-radiolabelled probe technology has been successfully applied to dot-blot analysis, colony hybridisation, _in situ_ and Southern blot hybridisation. Investigations into the sensitivity and reproducibility of the results are currently being done in by other laboratories (McKeating

Figure 12

The structure of Photobiotin.



et al., 1985), and results indicate the need for caution in the use of some of these commercially available reagents (Forster et al, 1985).

Aims

The aim of this study was two-fold:

(i) To examine variation in the DNA of HSV isolates from patients presenting at the Genitourinary Medicine Clinics and the Nuffield Transplant Unit, Edinburgh.

(ii) To assess the reproducibility, specificity and sensitivity of biotin and photobiotin, non-radiolablled nucleic acid hybridisation systems.

MATERIALS AND METHODS

Cell Culture and Media

(i) Human embryo fibroblasts (HEF) prepared in the Department of Bacteriology and a continuous line of Vero cells were grown in Eagle's minimal essential medium (MEM) (Cruickshank et al., 1975) supplemented with 5% (v/v) heat-inactivated newborn calf serum (NBCS) obtained from Flow Ltd.

(ii) Bacterial host LE3952 obtained from the Department of Genetics, was grown in NZCYM medium (Maniatis et al., 1982).

Standard Reference Strains

(i) Viral strains 17syn^{+} (HSV 1) and HG52 (HSV 2) were kindly supplied by the Institute of Virology, University of Glasgow.

(ii) Adenovirus 2 stock was obtained from Dr N Maitland (formerly) Department of Genetics, University of Edinburgh.

(iii) Bacteriophage λ (Charon 4A) was kindly supplied by J Kinross, Department of Genetics, University of Edinburgh.

Cloned DNA

(i) Cloned HSV DNA (EcoRI-O) and vector pBR322 were supplied by Dr N Maitland, University of Edinburgh.

(ii) Cloned HPV DNA (2 and 4) were kindly provided by Dr S Burnett, University of Birmingham.

(iii) Cloned HBV DNA (8-1-37) was a gift from Professor K Murray, University of Edinburgh.

(iv) Cloned CMV DNA (HindIII-1) was supplied by the PHLS Laboratory, Porton Down.

Patients

(i) 371 clinical isolates of genital HSV infections were obtained from 302 patients attending either the Department of Genito-urinary Medicine (GUM) of the Royal Infirmary, Edinburgh, or the South Fife Hospitals. The clinical details of these patients were provided by Dr G Scott (GUM).

(ii) Twenty seven oral HSV samples from renal transplant recipients (RTR) were isolated by Dr E Edmond (University of Edinburgh) during the course of a CMV study. All patients attended the Nuffield Transplant Unit, Edinburgh.

(iii) Fifteen HSV isolates from the London area were kindly supplied by Professor Banatvala, St Thomas's Hospital, London.

Serology

(a) Typing of isolates

The HSV isolates were typed by indirect immunofluorescence as described by Maitland *et al*. (1982). (See page 58)

(b) Immunoblotting technique

The serology of selected patients was determined in this laboratory by Dr P Simmonds. (Simmonds, Smith and Peutherer 1987 Journal of Medical Virology, in press) (See page 58).

(i) Typing of HSV isolates

Antiserum for typing was prepared in rabbits by immunisation with HSV 1 strain 1657 (Peutherer J.F. 1970. Journal of Medical Microbiology 3: 267-272). The serum was adsorbed three times with vero cells infected with MS strain of HSV 2. The results were visualised on a Leitz SM Lux microscope with incident UV light. Infected cells which reacted equally with the unadsorbed and adsorbed antisera were typed as HSV 1, whereas those reacting only with the unadsorbed sera were typed as HSV 2.

(ii) Immunoblotting

Vero cells, both uninfected and infected with HSV 1 (strain 1657) or HSV 2 (strain MS) were prepared then lysed with SDS-mercaptoethanol. The solubilised HSV proteins were then separated for 4.5 hrs (160v, 25mA) on 10% polyacrylamide gels with NN-methyline bis acrylamide cross-linker. The polypeptides were electrophoretically transferred to nitrocellulose which was blocked with 3% gelatin - Tris buffered saline, then cut into strips. The strips were reacted with the patient's serum and bound antibody was detected by incubation with horseradish peroxidase conjugated anti human serum. The substrate was 4-chloro-1-napthol obtained from Biorad.

Isolation and Viral Propagation

(i) Isolation of clinical specimens

All viruses were isolated in Human Embryo Fibroblast Cells (HEF) by the Virus Diagnostic Laboratory, University of Edinburgh.

(ii) Viral propagation

Viruses were passed to Vero cells grown in MEM (Cruickshank *et al.*, 1975) and used for DNA preparation within passage numbers four to eight. For each virus isolate two sub-confluent Roux bottles of Vero cells ($\sim$50 x 10^6 cells/Roux) were prepared and inoculated at a multiplicity of infection (MOI) of approximately 0.1 PFU/cell in 20mls of MEM. After adsorption for 2 hours at 37°C, a further 30mls of MEM was added and incubation allowed to continue until a complete cytopathic effect (CPE) was observed ($\sim$16 hours). The infected cells were subsequently harvested with glass beads, washed twice in Dulbecco A (Cruickshank *et al.*, 1975) and stored as a pellet (-70°C) until used to prepare HSV DNA (Maitland *et al.*, 1982).

(iii) Microcarrier cell culture

Bulk preparations of the standard strains HG52 and 17syn+ were grown using CYTODEX 1 microcarriers (Pharmacia). Three grams of microcarrier beads were prepared (as described by the manufacturers) then seeded with 4 x 10^7 Vero cells in 250mls of prewarmed (37°C) MEM. The culture was allowed to settle (with occasional stirring) for four to five hours when a further 250mls of MEM was added and continuous stirring allowed to commence (5-10rpm). A final 500mls

of MEM was added after two days and replenished in three days time with fresh (500mls) MEM. The cells were allowed to grow until confluent (~6 days) whereby the microcarriers were washed in serum free MEM then suspended in a minimal amount of MEM containing 1% (v/v) NBCS before HSV was added at approximately 0.1 PFU/cell. After adsorption for 2 hours (37°C, occasional stirring), the culture volume was increased to 500mls with MEM containing 10% (v/v) NBCS, glucose (2g/l) and non-essential amino acids (as described by Pharmacia). A further 500mls of MEM (supplemented as above) was added after 3 days. The culture was stirred until a CPE was observed (~6 days) then the MEM was removed and the infected microcarriers were washed in Dulbecco A. The infected cells were removed by a (1:1) mixture of trypsin (0.1%) - versene (0.02%) - Dulbecco A solution (pH 7.6) (15 mins, 37°C), then harvested as recommended by the manufacturer. The cell pellet was washed and stored at -70°C as previous described.

(iv) Growth and preparation of Adenovirus 2 DNA was as described by Maniatis *et al* (1982).

Preparation of HSV DNA

Cell pellets were resuspended in TE (0.01M Tris - 0.01M EDTA, pH 8) buffer and treated with SDS-Pronase as described by Maitland *et al*. (1982). The lysates were prepared for density gradient ultracentrifugation by adjusting the final volume to 8mls with TE containing 0.66% SDS and 80ug/ml of ethidium bromide. Sodium iodide (7.5g) and sodium metabisulphite (50mg/ml) were then added and the mixture vortexed before centrifugation at 500g for 10 mins. The

protein-SDS pellicle was then removed and the solution vortexed before being evenly distributed into 'Quick-Seal' (Beckman) centrifuge tubes. These were subsequently placed in a VT1-65 rotor and spun at 50,000rpm in a Beckman L8-55 ultracentrifuge. Viral DNA (see Fig 13) was harvested under ultraviolet illumination, by side puncture of the centrifuge tube. The DNA was extracted as described by Maitland *et al.* (1982) then resuspended in TE buffer, pH 8.

Restriction Endonuclease Digestion

Approximately 1ug of viral DNA from all HSV 1 and HSV 2 isolates was digested with a ten-fold excess of the endonucleases *EcoRI*, *BglII*, *HindIII*, *KpnI*, *BamHI*. In addition, *PvuII* and *HpaI* were occasionally used for HSV 1 isolates. All endonucleases were purchased from NBL Enzymes Ltd. Reaction buffers were as described by Maniatis *et al.* (1982) except for *KpnI* and *HpaI* which were prepared as recommended by the manufacturers. All samples were incubated at 37°C for a minimum of 2 hours when the reaction was stopped by the addition of 1/5th of the volume of loading dye (0.25% bromophenol blue, 0.15% Ficoll in TE, pH 8).

Agarose gel electrophoresis

(i) Restricted DNA fragments were separated on 0.4, 0.6 and 0.8% agarose (Sigma Type II EEO) gels containing 1ug/ml of ethidium bromide. The gels were cast in a horizontal apparatus (40cm x 20cm) and electrophoresed for 16-30 hours at 60v (32mA) in (0.04m) Tris - (0.005M) sodium acetate - (0.001M) EDTA (pH 7.9) buffer. The gels

were viewed under short wave ultraviolet transillumination and photographed with a Polaroid MP5 camera fitted with a Wratten #22 filter.

(ii) Agarose mini-gels (0.8 and 1%) were used for the rapid assessment of DNA concentration and the completion of RE reactions. The gels were cast on glass slides (8cm x 8cm) sealed with autoclave tape, and run in sandwich box tanks at 60v/100mA for 1-2 hours. The buffer was as described above.

Transfer of DNA from agarose gels

DNA was transferred either to nitrocellulose (Schleicher and Schull) or Genescreen (NEN) membrane following the method of Wahl *et al.* (1979), a modification of the Southern technique (1975). After overnight transfer, the membranes were rinsed in 2 x SSC and baked between sheets of Whatman 3mm paper for 2 hours at 80°C *in vacuo*.

Estimation of mobility variations

Mobility variations among analogous HSV DNA fragments were measured from photographs of ethidium bromide stained agarose gels. Measurement was carried out by using a magnifying lens (x 1.5) fitted with an internal microscale. The mobility differences were estimated relative to fragments of known molecular weights.

Analysis of RE data

Analysis of RE site distribution was carried out on an IBM personal computer, using dBase III.

Preparation and purification of DNA for probes

(i) Fragments of HSV DNA were either electroeluted into preformed troughs as described by Maniatis et al. (1982), or cut from the gel and placed in dialysis tubing. The dialysis tubing containing the gel fragments and TE buffer (pH 8) was placed in a mini-gel tank and electrophoresed for 2 hours at 100v. The current was then reversed for 2 mins allowing the DNA on the inside wall of the dialysis tubing to come into solution. The solution containing the DNA was filtered through a glass-wool column in order to remove any agarose fragments. The DNA solution was then extracted with phenol-chloroform and precipitated overnight with ethanol at -20°C.

(ii) HSV DNA of standard strains or DNA extracted from gel fragments was purified (as above (i)) by using a 'NACS PREPAC' affinity mini-column (BRL Ltd). The buffer systems for binding and elution were as recommended by the manufacturer. The eluate was diluted two-fold with an equal amount of distilled water prior to ethanol precipitation at -70° for 10 mins. The samples were centrifuged at 12,000g for 15 mins to collect the DNA pellet. This was subsequently washed with 70% ethanol and dried under vacuum before being resuspended in distilled water.

Radioactive labelling of DNA probes

Either standard strain HSV DNA or DNA fragments electroeluted from agarose gels were radiolabelled according to the method of Rigby et al. (1977). Approximately 0.25ug of purified HSV DNA was

mixed with 20uM of dNTP and 2uM (20uCi)(α-^{32}P) labelled dCTP (Amersham International) in 40ul of reaction mixture containing 50mM KPO_4 (pH 7.4), 5.2mM $MgCl_2$, 4mM dithiothreitol and 17.5ul of distilled water. E. coli DNA polymerase I (8 units) (purchased from NBL Enzymes Ltd) was added to the reaction mixture and incubated for 1 hour at 15°C. The reaction was terminated by the addition of 2ul of 0.25M EDTA, and the final volume of the DNA probe was made up to 0.1mls with TE buffer (pH 8). Unincorporated nucleotides were separated by centrifugation through a Sephadex G-50 (Pharmacia) column prepared as described in Maniatis et al. (1982). The specific activity of the DNA probe was measured using a Liquid Scintillation Counter.

Before hybridisation, the ^{32}P-probe was denatured in a boiling water bath (5 mins), chilled briefly, and made up to 10mls with 6 x SSC, 10% dextran sulphate (Pharmacia), 25% formamide, 2.5mM EDTA, 0.5% SDS and 1 x Denhardt's Solution (Denhardt, 1966).

Hybridisation of Membrane Filters

The baked membrane filters were preincubated with 10 x Denhardt's Solution (Denhardt, 1966) in a heat-sealed polyethylene bag. After prehybridisation (65°C for 4 hours) in a shaking water bath, the Denhardt's solution was removed and replaced with the ^{32}P-labelled probe mixture (4-5 x 10^5 cpm/ml). The bag was resealed and hybridisation allowed to continue for 16 hours at 68°C in a shaking water bath. The filters were washed twice for a period of 2 hours in 2 x SSC (64°C) and dried for 1 hour at 80°C in vacuo.

Autoradiography

Autoradiography of the hybridised membrane filters was carried out for 16 hours - 2 days at -70°C. The films (Kodak X-omat S) were pre-flashed before use in autoradiography cassettes fitted with Kodak X-Omatic regular intensifying screens.

Polyacrylamide gel electrophoresis

Viral DNA was digested with either BamHI or KpnI as previously described. After the addition of 10ul of loading dye (25% w/v sucrose, 0.2% bromophenol blue) the digests were loaded on to a 3% (w/v) polyacrylamide stacking gel with a 7.5% (w/v) separating gel (14 x 19 x 0.15cm) containing 0.05% w/v sucrose. Electrophoresis was carried out at 50v until the samples entered the separating gel, when the voltage was increased to 70v (22mA) for 16 hours. The buffers and conditions were as described by Herring *et al* (1982). Molecular weight standards (Ø x 174RF/HaeIII digests and/or λ/HindIII digests) were included in each gel.

Silver staining of polyacrylamide gels

The gels were stained using a modified version of the technique described by Herring *et al*. (1982). After removal from the glass plates, the gel was washed at 20 min intervals with three changes of 10% ethanol - 0.5% acetic acid (500mls). The gel was then soaked for 20 mins in 200mls of 0.11M silver nitrate solution and rinsed rapidly in 500mls of distilled water prior to development in 200mls of 0.75M sodium hydroxide, 0.1M formaldehyde solution. Development

was allowed for a maximum of 10 mins after which the gel was enhanced in 500mls of 0.07M sodium carbonate solution (15 mins). The gel was subsequently placed in a further 500mls of fresh carbonate solution (2 hrs) before being removed and stored in heat-sealed polythene bags. The results of each gel were photographed by transmitted light with a Pentax ME camera.

Non-radiolabelled DNA detection systems

(i) BRL DNA Detection System

(a) Preparation of DNA test strips

A stock solution of biotinylated DNA (M13) was diluted with buffer (6 x SSC, 0.2ug/ul sheared herring sperm DNA) to final concentration of 10pg/ul, 4pg/ul, 2pg/ul and 1pg/ul. Five ul of each dilution and 5ul of dilution buffer were sequentially spotted on nitrocellulose strips (1 x 5cm) then dried for 1-2 hours at 80°C under vacuum. The test strips were stored dry until used to monitor the sensitivity of each DNA detection experiment.

(b) Preparation of biotin-labelled DNA probes

Purified DNA was labelled by nick translation in the presence of biotin-11-dUTP as described by Langer et al. (1981). As recommended by the manufacturer, the BRL nick translation reagent system was used in the quantities and conditions described in the accompanying handbook. Once labelled the biotin-DNA probe was separated from unincorporated nucleotides by gel filtration on a 5ml Sephadex G-50 (Pharmacia) column. The column was equilibrated with 1 x SSC/0.1% (w/v) SDS before 150ul fractions of the biotin-labelled

DNA were collected. Two ul of each fraction was spotted on nitrocellulose strips and dried in a vacuum even at 80°C for 1-2 hours. Fractions containing labelled DNA were detected by colour development (as described in (d)) and pooled for use in hybridisation.

(c) Hybridisation

Conditions were similar to those for radiolabelled probes and have been described in detail by Leary *et al*. (1983). The essential changes are in the reduction of the amount of formamide to 45% and the low hybridisation temperature (42°C). After hybridisation the filters were washed twice in 2 x SSC - 0.1% (w/v) SDS (250mls) for 3 mins at 20°C, then once in 0.2 x SSC - 0.1% (w/v) SDS. The filters were subsequently incubated in two changes of 250mls 0.16 x SSC - 0.1% SDS at 50°C for 15 mins each time. After a brief wash in 2 x SSC - 0.1% (w/v) SDS (20°C) the filters were introduced to the detection system (see (d) and (e)).

(d) Detection system

The filters were incubated at 42°C for 20 mins in solution A (0.1M Tris-Cl (pH 7.5), 0.1M NaCl, 2mM $MgCl_2$ and 0.05% (v/v) Triton X-100) containing 3% (w/v) BSA. After incubation the filters were dried under vacuum (10-20 mins, 80°C) then rehydrated in the above solution before being incubated in BRL streptavadin (6ug/100cm^3 nitrocellulose) for a further 10 mins. The streptavidin was removed and the filters washed (3 x 2 mins) in solution A before being incubated in BRL poly (AP) (3ug/100cm^2 nitrocellulose) for 10 mins, then washed (2 x 2 mins) in solution A. A final washing step (2 x 2

mins) was performed with solution B (0.1M Tris-Cl (pH 9.5), 0.1M NaCl, 50mM $MgCl_2$) before the visualisation process.

(e) Visualisation

Dye solution (NBT and BCIP in solution B) was prepared as recommended by the manufacturer and sealed with the filter in a polypropylene bag. The incubation was performed at room temperature in a dark cupboard and allowed to continue for a maximum of 4 hours. To terminate the reaction the filters were washed in 20mM Tris (pH 7.5) - 5mM EDTA, then blotted dry and stored. A test strip was used for each reaction to monitor the colour development.

(ii) Photobiotin Acetate System (BRESA Ltd)

(a) Preparation of the DNA probe

Purified DNA (0.5-1ug/ul) was combined with an equal volume of photobiotin acetate (1mg/ml) in an unsealed microfuge tube, then placed in a tray of crushed ice. The samples were then irradiated for 20 mins at 10cm beneath two Crompton 250 watt mercury-tungsten lamps. After irradiation the reaction mixture was made up to a final volume of 100ul with 100mM Tris-Cl, 1.0mM EDTA (pH 9), then extracted twice with an equal volume of butan-2-ol. DNA was recovered by the addition of 5ul (3M) sodium acetate and 100ul of ethanol (-20°C). After 16 hours the solution was centrifuged in a micro-centrifuge (15 mins, 4°C) to yield the labelled DNA pellet. The pellet was washed in 70% (v/v) ethanol, dried *in vacuo* and resuspended in 0.1mM EDTA. Probes were either used immediately or stored at -20°C.

(b) Hybridisation

The hybridisation conditions used have been described in detail by Forster et al (1985).

(c) Colorimetric detection

The filters were blocked with BSA solution and treated with avidin-alkaline phosphatase (under the conditions decribed by BREAS Ltd) then washed (in solution A) to reduce non-specific binding and added to the substrate solution (NBT-BCIP) for a maximum of 4hours. The reaction was terminated in 10mM Tris-Cl, 1.0mM EDTA (pH 7.5) and the filter blotted dry before being stored. A photobiotin-labelled DNA control (M13) was spotted on each filter and used to monitor the colour reaction.

Preparation of Viral DNA for Use with Photobiotin

(i) HSV DNA (HSV 1 and HSV 2) was prepared and purified as previously described.

(ii) CMV DNA prepared from urine specimens (Chou and Merigan, 1983) was kindly supplied by Dr E Edmond.

(iii) HPV DNA was prepared from frozen wart specimens (supplied by the Department of Dermatology, University of Edinburgh) by a modification of the method of Rudlinger et al. (1986). The warts were resuspended in 1ml of Hirt buffer to which 100ug of predigested pronase (Sigma) was added. The warts were then incubated (37°C, 2 hours) before the addition of a further 100ug of pronase. After overnight incubation (37°C) 1/5th of the volume of 5M NaCl was added and the samples held at 4°C for 10 mins (12000g) and the supernatant

removed to a glass tube. After three extractions with phenol-chloroform (1:1) the DNA was precipitated overnight with ethanol (-20°C). The DNA pellet was then washed in70% ethanol (3 times), dried under vacuum and resuspended in TE buffer (pH 8). The cloned HPV DNA for probe preparation was amplified by the method of Birnboim and Doly (1979) then the DNA precipitated at -20°C (16 hours) with ethanol.

(iv) HBV DNA was prepared from serum samples supplied by Dr JF Peutherer, Hepatitis Reference Laboratory, University of Edinburgh. The procedure used (a modification of Landers et al. 1977) was carried out at 4°C on a Spinco-L centrifuge. Seven mls of serum were clarified by centrifugation for 10 mins at 2,000rpm (SW40 rotor). Five mls of the supernatant was then removed and combined with 5mls of TNEMBSA buffer. The mixture was then centrifuged for 2 hours (SW40 rotor, 35,000rpm, 4°C) to pellet the DANE particles. The pellet was resuspended overnight (200ul TNEMBSA, 4°C) before being layered on a 20% sucrose gradient and spun at 40,000rpm for 2 hours (SW40 rotor, 4°C). The pellet was again resuspended overnight (100ul 0.01M Tris - 0.1M NaCl (pH 7.5), 4°C) then pelleted through a 30% sucrose gradient at 42,000rpm for 3 hours (SW50 rotor, 4°C). After resuspension in 100ul 0.01M Tris - 0.01M EDTA, pH 7.5 (4°C overnight), the pellet was incubated (37°C) with (2mg/ml) protease and 0.6% SDS for 90 mins. The preparation was subsequently phenol-chloroform extracted (3 x) and ethanol precipitated at -20°C for 72 hours. The DNA was collected, vacuum dried, and resuspended in TE (pH 8) buffer.

TNEMBSA = 0·1M Tris
0·01M EDTA
0·05M NaCl
0·01M $MgCl_2$
0·1% BSA

RESULTS

1. Microcarrier Cell Culture

The use of CYTODEX 1 microcarriers (see Methods) was investigated as a method for the production of bulk preparations of standard strain (HG52 and 17 syn$^+$) HSV DNA. A summary of the time for preparation and yield of HSV DNA produced by microcarrier cell culture compared with that of the more conventional production in Roux bottles is shown in Table 4.

Growth of vero cells in Roux bottles was carried out routinely in the laboratory and required a total volume of 300mls of MEM. HSV was added at a multiplicity of infection (MOI) of 0.1pfu/cell giving a yield of 1 x 10^{-7}ug of HSV DNA per cell (Table 4).

Growth of vero cells on microcarrier beads (Fig 14) was carried out under the conditions recommended by the manufacturers (see materials and methods) and required a total volume of 3 litres of MEM. Using the same MOI as in Roux bottle culture (0.1pfu/cell) produced a cytopathic effect (CPE) in approximately 6 days. During this time a number of vero cells had become detached from the beads, however 6 days was the minimum time for a good CPE to be produced. The yield of HSV DNA after harvesting was found to be approximately 6.25 x 10^{-3}ug/cell.

In an attempt to reduce the time taken to produce a good CPE by microcarrier culture an MOI of 1pfu/cell was used. In this instance a good CPE was observed after 5 days, however purification of the DNA on a sodium iodide gradient (Fig 13) revealed a large band of defective DNA that was unable to be separated from the HSV DNA. (Walbloomers and ter Schegget, 1976).

Figure 13

Sodium iodide density gradient showing separation of cellular and HSV DNA.

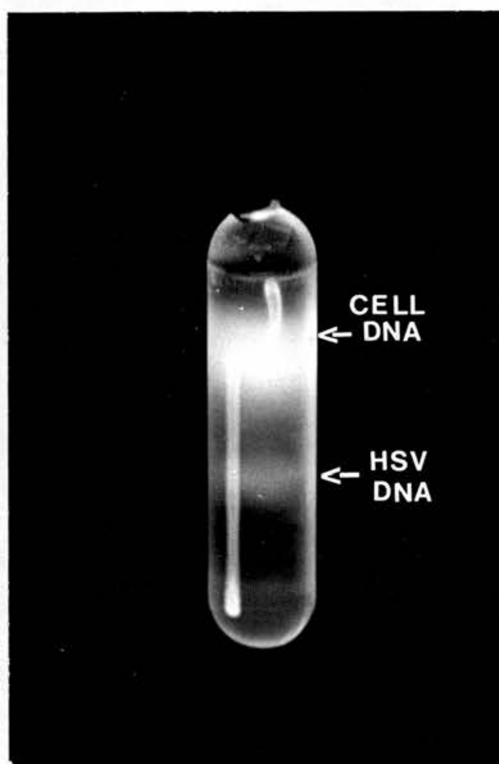


Figure 14

Vero cells one day after inoculation onto Cytodex microcarriers.
(X 120)

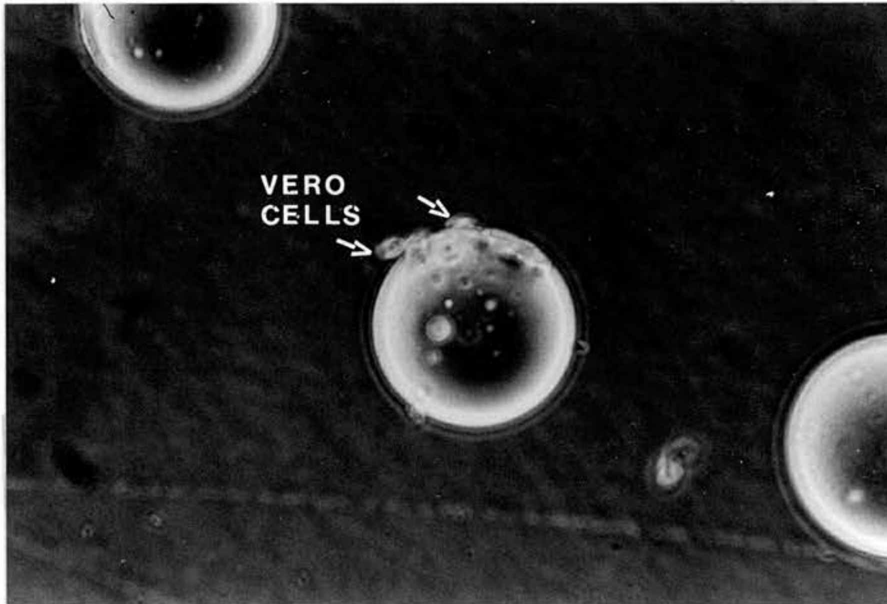


Table 4

Comparison of cell culture methods and yield for the production of HSV DNA

Method of cell culture	Total no cells required for seed	No of cells at time of virus inoculum	Time taken for production of CPE	Yield of HSV DNA/ cell	Total media requirement
2 Roux bottles	12×10^6	10×10^7	16 hrs	1×10^7	300mls
Microcarrier culture	4×10^7	40×10^7	6 days	6.25×10^6	3 litres

The method of microcarrier cell culture was discontinued due to the expense of the media and the time required for production of DNA.

2. The reproducibility of agarose gel electrophoresis with standard laboratory strains

In order to determine the reproducibility of the technique, preparations of standard strain DNA (HG52 and $17syn^+$) were prepared (as described in Materials and Methods), digested to completion with appropriate restriction enzymes then separated by agarose gel electrophoresis. The experimental design was as follows:

(a) Standard strain DNA (HG52 and $17syn^+$) prepared from a single preparation was examined on a number of occasions to allow determination of reproducibility.

(b) Standard strain DNAs from 3 separate preparations were repeatedly run in parallel tracks on agarose gels, thus allowing a comparison of both reproducibility and variation between consecutive DNA preparations.

In both cases (a and b) the overall restriction endonuclease profile remained constant, with no major variations observed between preparations or on successive electrophoresis. Small mobility differences were noted in fragments BamHI g (HG52) and k ($17syn^+$) that occur in the joint region of the genome. The following studies therefore use only major variations as a criteria for distinguishing

HSV isolates. However, mobility differences have been noted and are discussed in section 3c and 4.

3. Analysis of DNA from Genital Isolates of Herpes Simplex Virus 1 and 2

(a) Patients

(i) Time of isolation of HSV 1 and HSV 2

During the 19 month period from 1st January 1982 to 31st July 1983, HSV was isolated from 224 patients with clinical evidence of herpetic infection. The patients were new attenders at the clinics held by the Department of Genitourinary Medicine (RIE) and South Fife Hospitals. The viruses were isolated and typed as described in the methods section, with 24.4% (44) found to be HSV 1 isolates. The monthly isolations of HSV 1 and HSV 2 are represented in Figure 15. Overall the number of first isolations for HSV 2 ranged from 3-22 per month, whereas those of HSV 1 ranged from 1-4 per month, having averages of 9.5 and 2.3 isolations per month respectively. A comparison of the isolations for the first seven months of each year showed only one notable difference in the month of June 1982 when 23 isolates (1 HSV 1, 22 HSV 2) were obtained. The peak isolation (22) of HSV 2 samples was recorded in July 1982, while in July 1982 the isolations of HSV 1 exceeded those of HSV 2.

(ii) Age and sex distribution

Consideration of age and sex distribution (Fig 16) demonstrates that for HSV 2 isolates the ages of the male patients ranged from 18

Figure 15

Isolation of HSV (types 1 and 2) from genital lesions during the period January 1982 - July 1983.

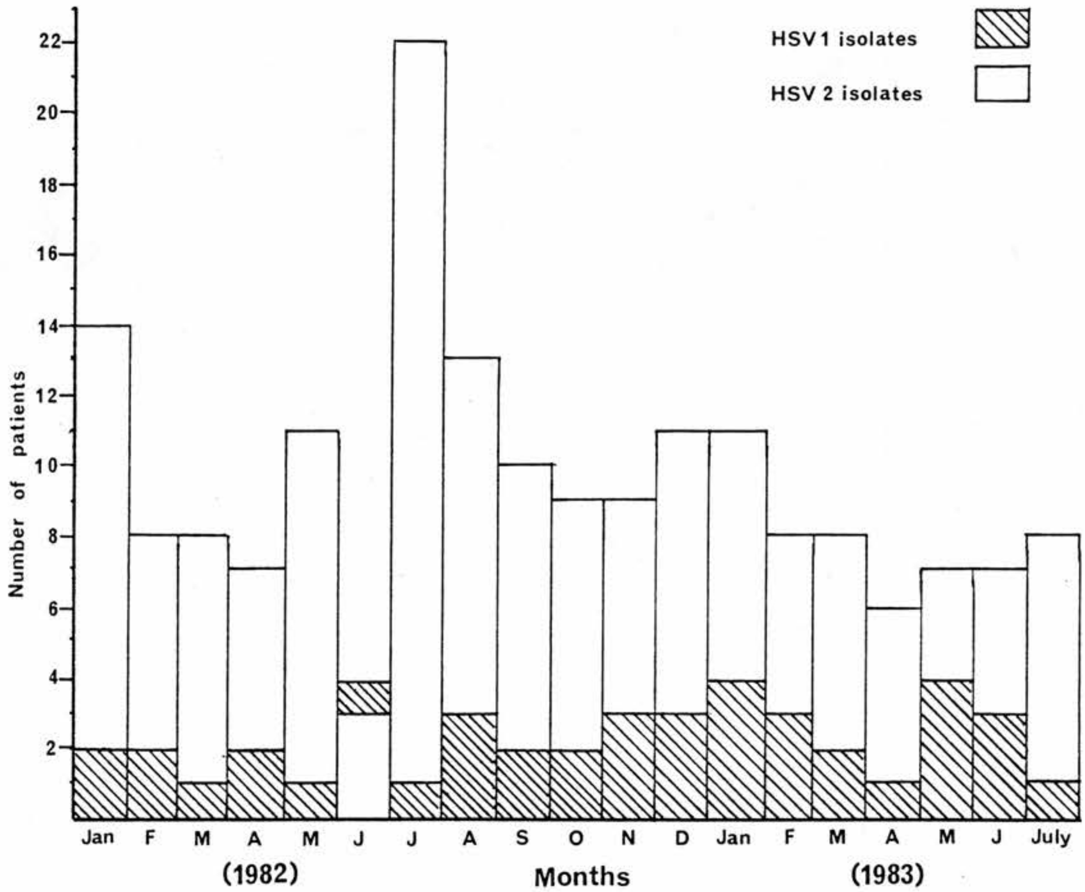
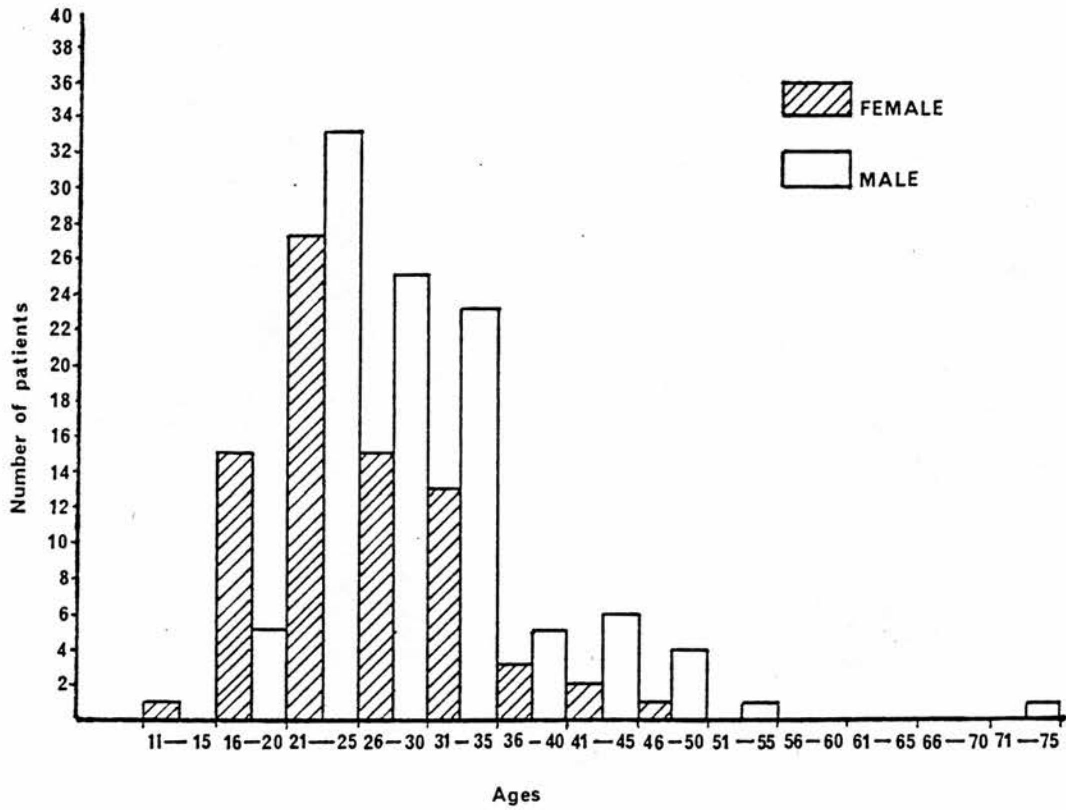


Figure 16

The age and sex distribution of patients with genital HSV 2.



to 72 years (average 31.2 years), while the female patients were aged from 14 to 47 years (average 25.2 years). Similarly for the HSV 1 isolates (Fig 17), the average age of the male patients was 25.6 years with a range of 18-35 years, whereas the female patients ranged from 18-37 years, having an average age of 22.3 years. The ratio of male to female patients for HSV 1 and HSV 2 was found to be 1:3.4 and 1:0.75 respectively.

(iii) Source of virus isolates

Virus was isolated mainly from lesions of the skin or mucous membranes of the genital tract with two exceptions, one from a urine sample, the other from lesions on the buttocks. The distribution of sites for virus isolation (Table 5) from male patients shows that the majority of HSV 2 samples were isolated from the prepuce (39.9%), for HSV 1 samples the prepuce and anus shared an equal number of isolates. In the majority of female patients HSV 2 was isolated from the cervix (25.9%), whereas the vulva was the most common isolation site (41.2%) for HSV 1 infections (Table 6). Further consultation of clinical data showed that 33 females were recorded as having HSV isolated concurrently from the cervix and another genital site.

Thirteen HSV 2 and 9 HSV 1 specimens whose recorded site of isolation was a genital site (as described in Table 6b) also had a positive cervical isolation. In addition, 11 specimens (10 HSV2, 1 HSV 1) whose recorded site of isolation was the cervix also had a concurrent genital isolation from an unspecified site. Despite adjustment for these figures, the vula was still found to be the

Figure 17

The age and sex distribution of patients with genital HSV 1

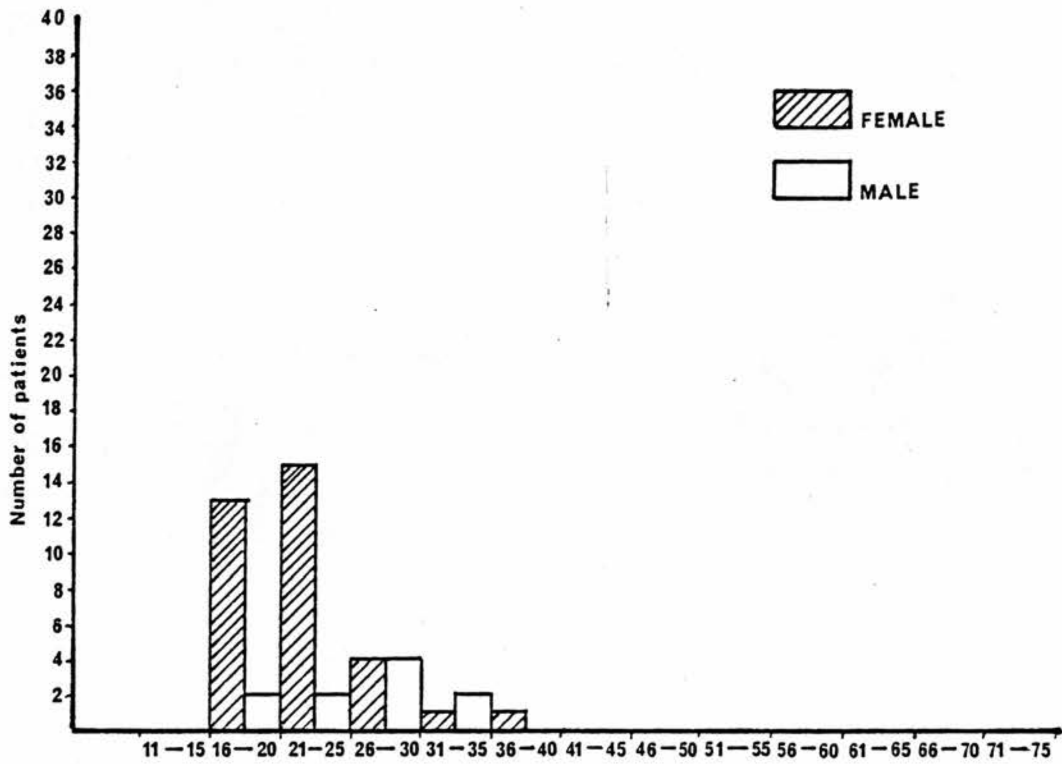


Table 5

Anatomical sites of isolation for male patients with genital HSV (103 HSV 2, 10 HSV 1).

Site of isolation (as detailed by the request form)	Number of HSV 2 isolations (%)	Number of HSV 1 isolations (%)
Penis	13 (12.6)	2 (20)
Glans	3 (4.8)	-
Coronal sulcus	11 (10.7)	-
Prepuce	41 (39.9)	3 (30)
Frenum	3 (2.9)	-
Urethra	8 (7.8)	-
Urine	-	1 (10)
Anus	5 (4.8)	3 (30)
Thigh	1 (1.0)	-
Buttocks	1 (1.0)	-
Not specified	15 (14.5)	1 (10)

Table 6

(a) Anatomical sites of isolation for female patients with genital HSV (77 HSV 2, 34 HSV 1)

Site of isolation (as detailed by the request form)	Number of HSV 2 isolations (%)	Number of HSV 1 isolations (%)
Cervix	20 (25.9)	5 (14.7)
Vulva	13 (16.9)	14 (41.2)
Labia	18 (23.4)	6 (17.7)
Fourchette	4 (5.2)	-
Introitus	7 (9.1)	1 (2.9)
Clitoris	3 (3.9)	1 (2.9)
Perineum	1 (1.3)	3 (8.8)
Anus	3 (3.9)	1 (2.9)
Not specified	8 (10.4)	3 (8.8)

(b) Number of female patients from whom HSV was isolated concurrently from genital (as 6a) and cervical sites.

Anatomical site of isolation	Number of HSV 2 isolates with positive cervical isolation	Number of HSV 1 isolates with positive cervical isolation
Vulva	4	3
Introitus	2	-
Labia	3	2
Clitoris	1	1
Fourchette	2	-
Perineum	-	2
Not specified	1	1
Total	13	9

10 HSV 2 specimens and 1 HSV 1 specimen recorded as having been isolated from the cervix also had a concurrent genital isolation.

6 patients recorded as having HSV 2 isolated from the cervix had negative genital isolations, while 13 patients with positive genital isolations had negative virus isolation from the cervix.

most common isolation site for HSV 1 infections in female patients. However, for HSV 2 isolations the cervix, vulva and labia may share equal numbers of successful HSV isolations from female patients.

(b) Restriction endonuclease analysis

The fragment nomenclature used throughout is that of Davison and Wilkie (1981) determined for the Glasgow standard strains 17syn$^+$ (HSV 1) and HG52 (HSV 2). All 224 HSV isolates were examined with five restriction endonucleases EcoRI, BglII, HindIII, KpnI and BamHI as described in the Methods section. Any new sites or fusions were initially determined by simple molecular weight additions. However hybridisation experiments were carried out (an example of which is shown in Fig 18) to verify the map positions of new sites or fusions and to enable distinction between major variations (Fig 8) and simple mobility differences (Figs 33, 34). The data obtained from the agarose gels (e.g. Figs 19-24) are summarised in Tables 7 and 8 for HSV 2 and HSV 1 isolates respectively. Map positions for the major RE variations are also represented in Figs 25 (HSV 2) and 26 (HSV 1). Specific RE data for each isolate is listed in the Appendix.

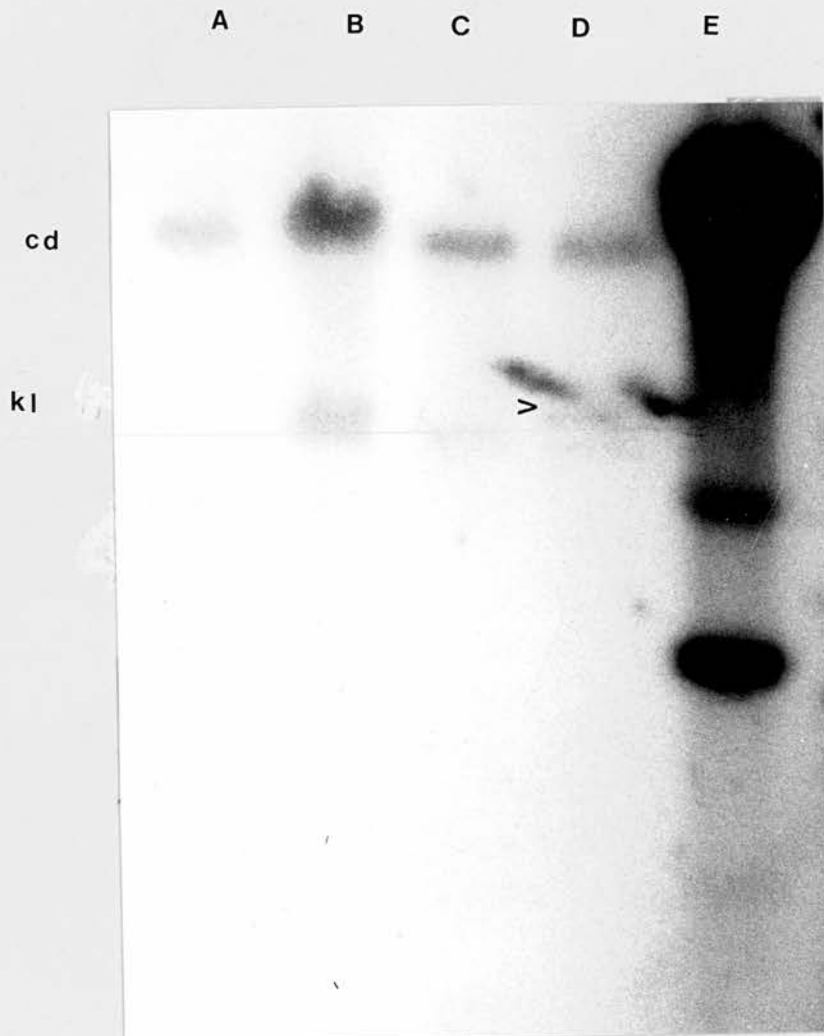
(i) Analysis of HSV 2 isolates

On the basis of addition or deletion of specific enzyme recognition sites the 180 HSV 2 isolates were divided into 18 groups as shown in Tables 9a and b. No further distinction could be made between the HSV 2 isolates purely on the basis of variable site combinations as identified with the five endonucleases.

Figure 18

Autoradiogram of HSV 2 DNA digested with HindIII and separated on a 0.4% agarose gel. Hybridisation with ^{32}P-labelled EcoRI-o fragment (see methods) confirmed the presence of an m-l fusion in track D.

Tracks A-C - prototype RE profile
Track D - m-l fusion
Track E - Adeno 2/HindIII



Prototype tracks A, B and C demonstrate hybridisation of HindIII fragments k, l, c and d with EcoRI-O. Track D, with an m-l fusion, shows hybridisation of an additional band (the fusion fragment) at 5.5kbp. The quarter molar fragment k is not easily visualised in this autoradiograph.

Figure 19

Agarose gel showing HindIII digests of HSV 2 DNA from genital isolates.

Tracks A, B - prototype RE profiles
Track C - m-l fusion

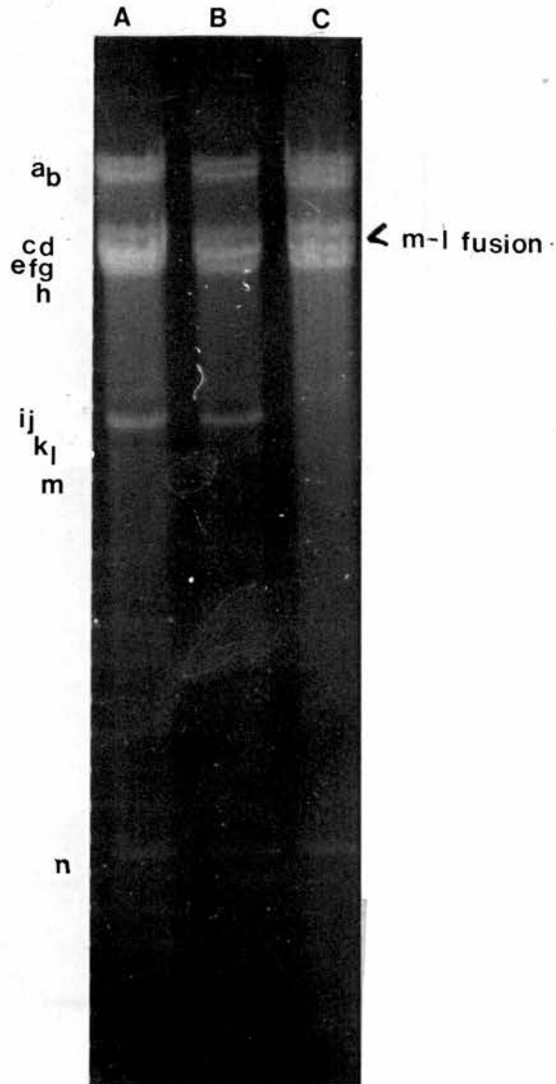


Figure 20

Agarose gel showing KpnI digests of HSV 2 DNA from genital isolates.

Tracks A, B - d-i fusion
Track C   - d-i fusion and new site in fragment m

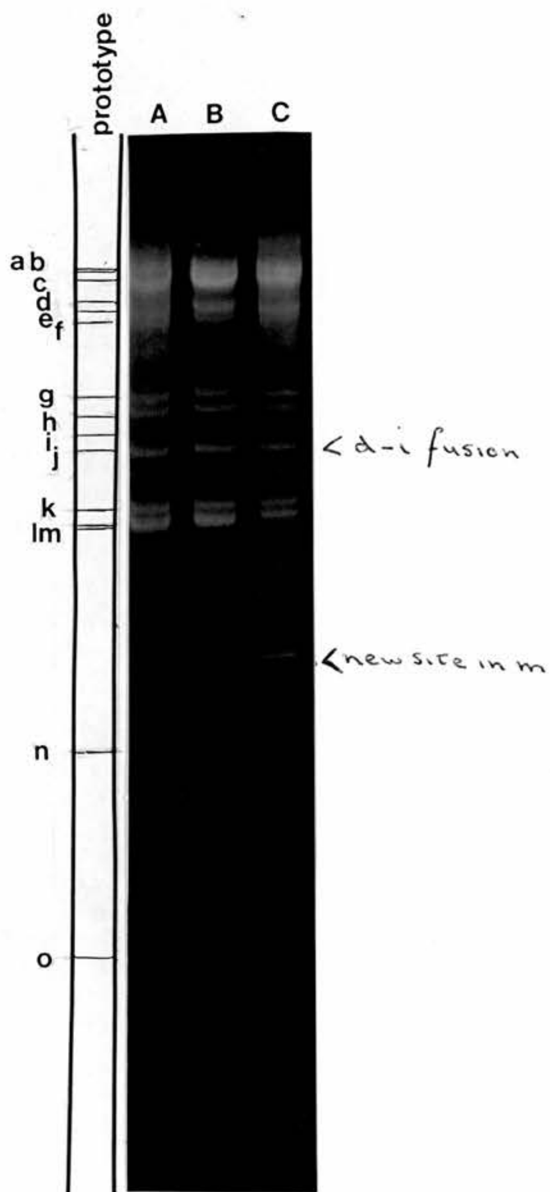


Figure 21

Agarose gel showing BglII digests of HSV 2 DNA from genital isolates.

Track A - prototype
Track B - p-r fusion

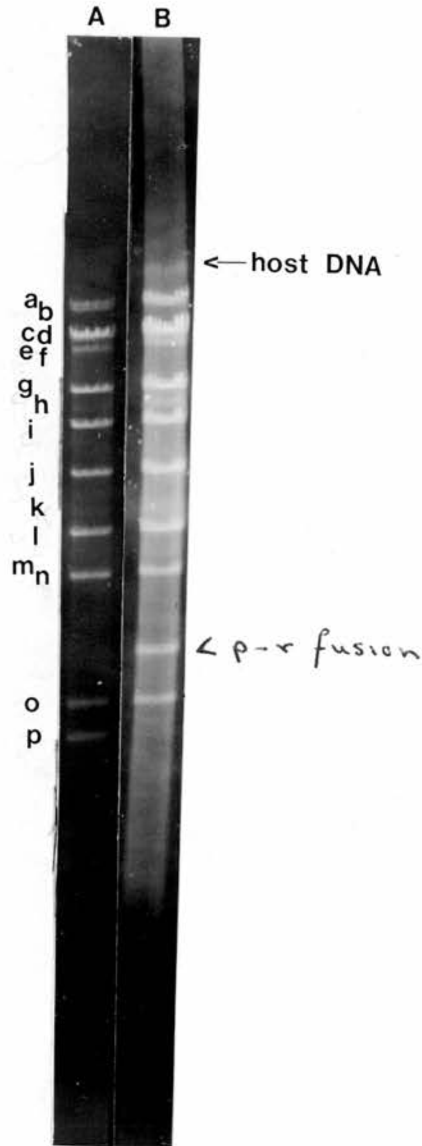


Figure 22

Agarose gel showing HindIII digest of HSV 2 DNA from genital isolates.

Track A - new site in fragment a (x)
Track B - new site in fragment e (o)

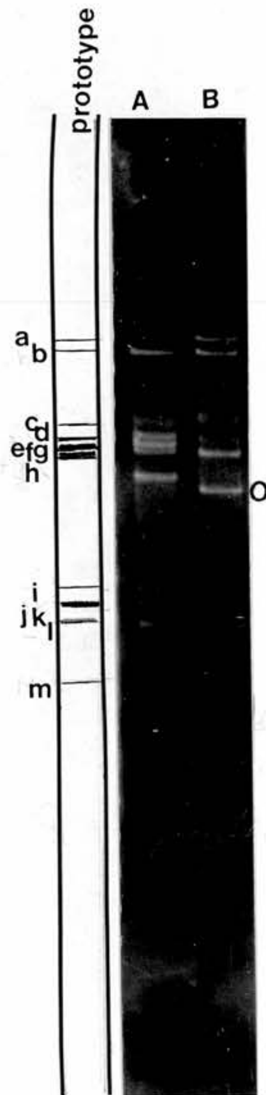


Figure 23

Agarose gel showing HindIII digests of HSV 2 DNA from genital isolates.

Track A - new site in fragment e
Track B - prototype

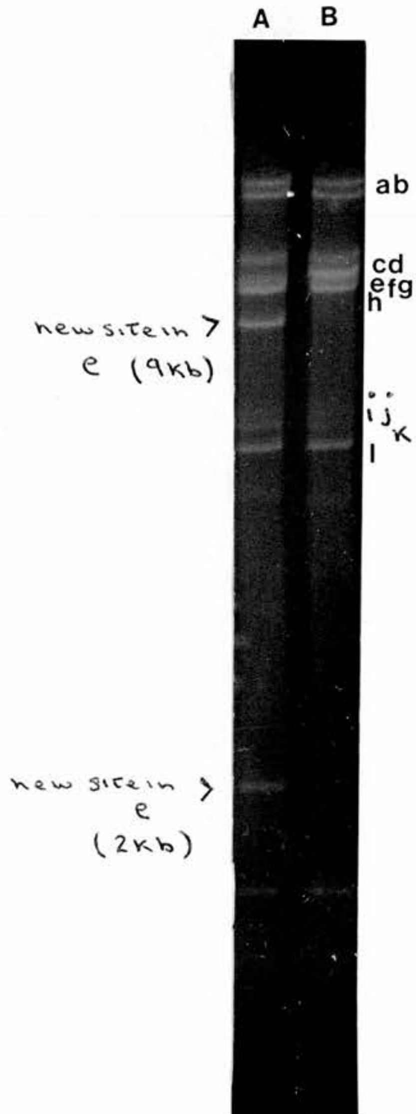


Figure 24

Agarose gel showing HindIII digests of HSV 1 DNA from genital isolates.

Track A - o-h fusion
Track B - prototype

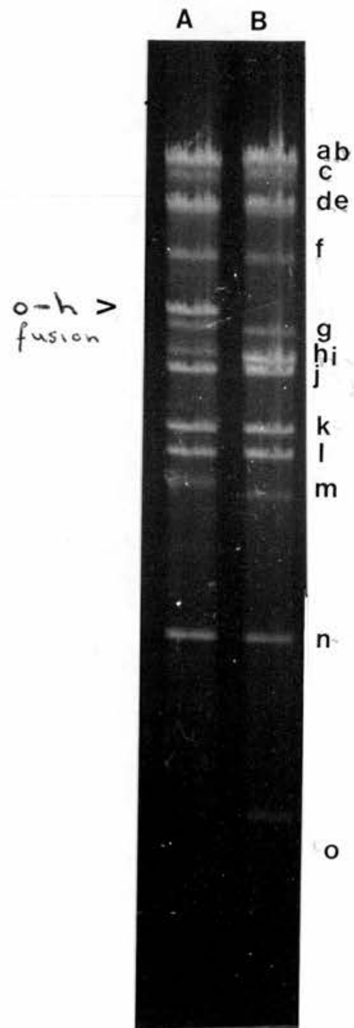


Figure 25

The map positions of variable sites in HSV 1 DNA as determined by restriction endonuclease analysis.

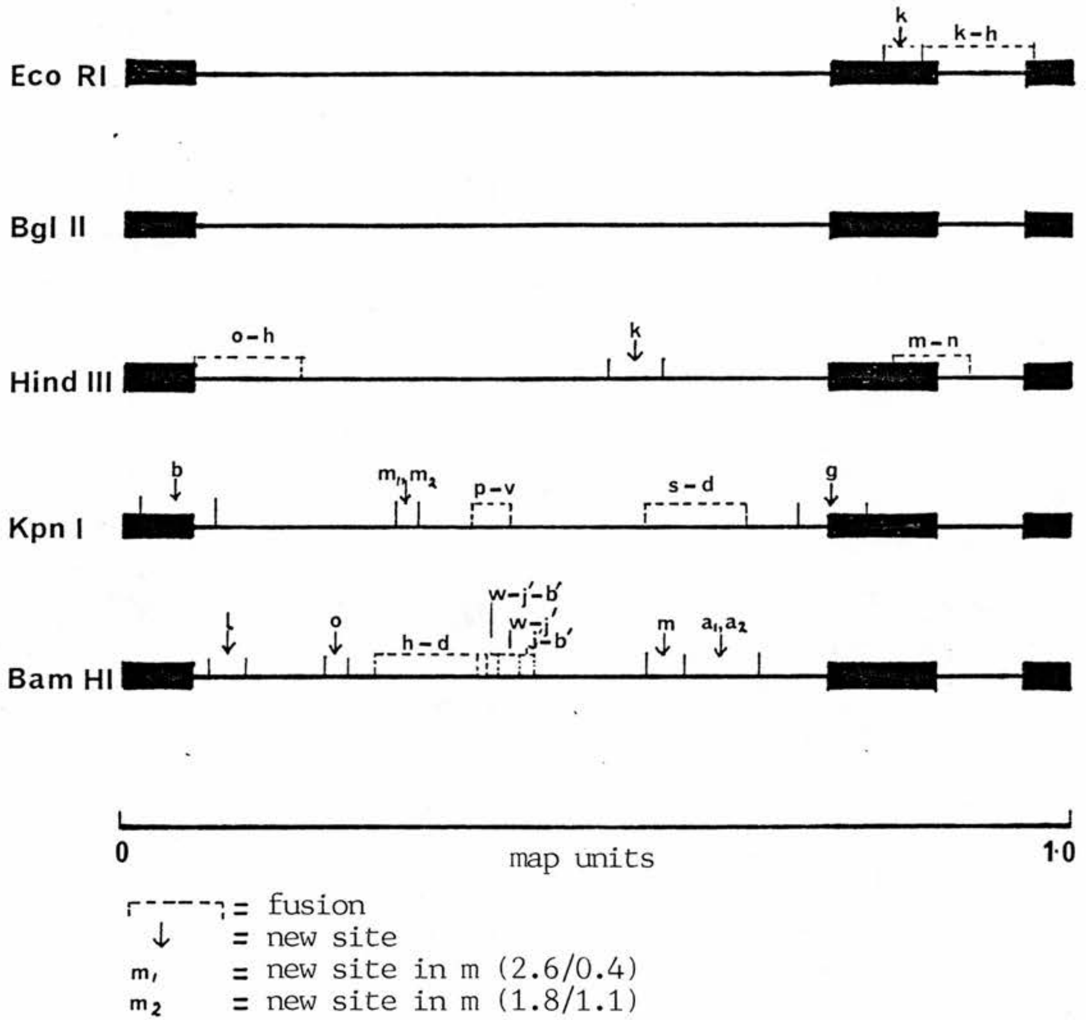


Figure 26

The map positions of variable sites in HSV 2 DNA as determined by restriction endonuclease analysis.

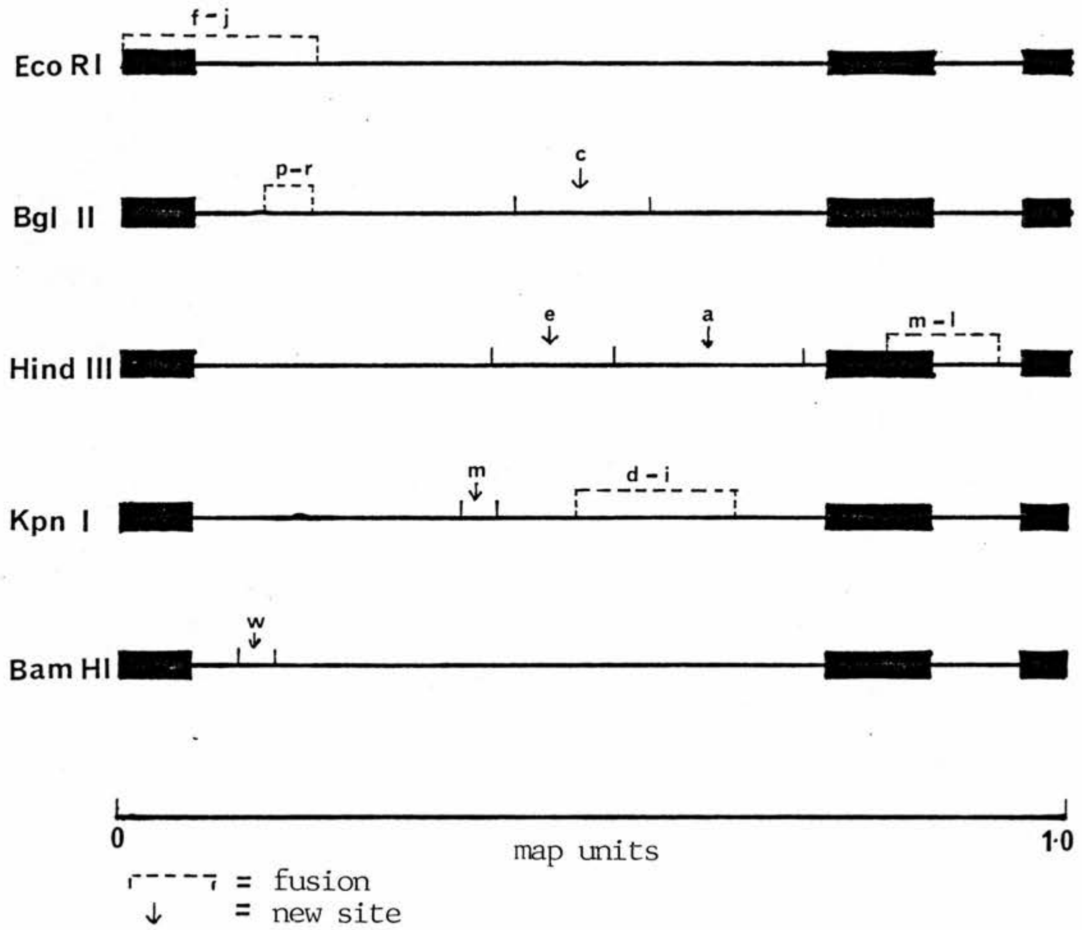


Table 7

Description and frequency of major variations in the restriction endonuclease sites of 180 genital HSV 2 isolates

Variable site		Number of isolates with variable site	(%)
EcoRI	f-j	81	(45)
BglII	c (13/3)	4	(2.2)
	p-r	1	(0.55)
HindIII	e (9/2)	21	(11.7)
	m-l	1	(0.55)
	a (11/9.5)	1	(0.55)
KpnI	d-i	147	(81.6)
	m (3.1/0.9)	1	(0.55)
BamHI	w (1/1)	79	(43.9)

Table 8

Description and frequency of major variations in the restriction endonuclease sites of 44 genital HSV 1 isolates

Variable site		Number of isolates with variable site	(%)
EcoRI	h-k	3	(6.8)
	k (2.0/1.5)	7	(15.9)
	l-a	1	(2.3)
HindIII	o-h	14	(31.8)
	k-l	1	(2.3)
	m-n	5	(11.4)
KpnI	s-d	42	(95.4)
	p-v	3	(6.8)
	b (6.2/1.8)	40	(90.9)
	g	38	(86.9)
	m (2.6/0.4)	13	(29.5)
	m (1.8/1.1)	9	(20.4)
BamHI	d-h	7	(15.9)
	w-j'	12	(27.3)
	j'-b'	6	(13.6)
	w-j'-b'	2	(4.5)
	a (7.2/0.8)	22	(50)
	a (5.8/2.2)	12	(27.3)
	l (1.7/1.8)	1	(2.3)
	m (2.8/0.4)	3	(6.8)
	o (2.4/0.2)	2	(4.5)

Table 9a

Variations in the restriction endonuclease profiles of genital HSV 2 (DNA) isolated between 1.1.82 - 31.7.83

Digestion patterns when restricted with: EcoRI BglII HindIII KpnI[a]	BamHI w[b]	Number of isolates
pppp	+	9
pppp	-	10
xppp	+	8
xppp	-	5
xpxx	+	3
xpxx	-	4
xppx	+	39
xppx	-	17
pxpx	+	1
pxpx	-	0
pppx	+	28
pppx	-	35
ppxx	+	9
ppxx	-	5
xxpx	+	3
xxpx	-	0

a = The order of digestion is, reading from left to right, EcoRI, BglII, HindIII, KpnI. Where p indicates prototype (as HG52), x indicates the following: for EcoRI, loss of f-j site; for BglII, extra site in c fragment; for HindIII, extra site in e fragment; for KpnI, loss of d-i site.

b = BamHI digestion pattern indicates presence (+) or absence (-) of fragment w.

Table 9b

Unique variable RE sites found in genital HSV 2 (DNA) isolated between 1.1.82 - 31.7.83

Isolate Number	EcoRI	BglII	HindIII	KpnI	BamHI
1465	P	P	P	m* d-i	(-)
12928	f-j	P	m-l	d-i	(-)
15762	P	P-r	P	P	(+)
7561	f-j	P	a	d-i	(-)

P = prototype
- = fusion between the restriction fragments as stated for each enzyme
a = new site in HindIII a fragment giving two new fragments of molecular weights 11 and 9.5Kbp
*m = new site in KpnI m fragment giving two new fragments of molecular weights 3.1 and 0.9Kbp
(-) = absence of BamHI w fragment
(+) = presence of BamHI w fragment

(ii) Analysis of HSV 1 isolates

All 44 HSV 1 isolates could be distinguished from each other solely by the criteria of site additions and deletions as identified with the five endonucleases. A detailed description of the variable restriction endonuclease sites is provided in Table 8.

(iii) Computer comparison of variable RE sites

A computer comparison (for both HSV 2 and HSV 1 isolates) of the frequency of RE site variation with sex (male vs female) or anatomical site of isolation (extra-genital sites vs genital sites) revealed no differences in either the frequency or the distribution of RE sites (data not shown). No correlation was found when the frequency of the variable sites recognised by the restriction enzymes were compared with the known functional regions of the genome.

(c) Fragment variability generated by restriction endonuclease digestion

During the course of the study the restriction patterns of digested HSV 1 and HSV 2 DNA revealed variation in the electrophoretic mobility of several fragments (Figs 27, 28). The mobility differences were recorded and the map positions of the variable fragments are shown in Figs 29 and 30 for HSV 1 and HSV 2 respectively. Most variation in both HSV 1 and HSV 2 isolates was found in the L-S junction and terminal fragments of the genome. The electrophoretic mobilities observed with BamHI L-S junction

Figure 27

Agarose gel showing mobility differences obtained by digestion of HSV 2 DNA with EcoRI.

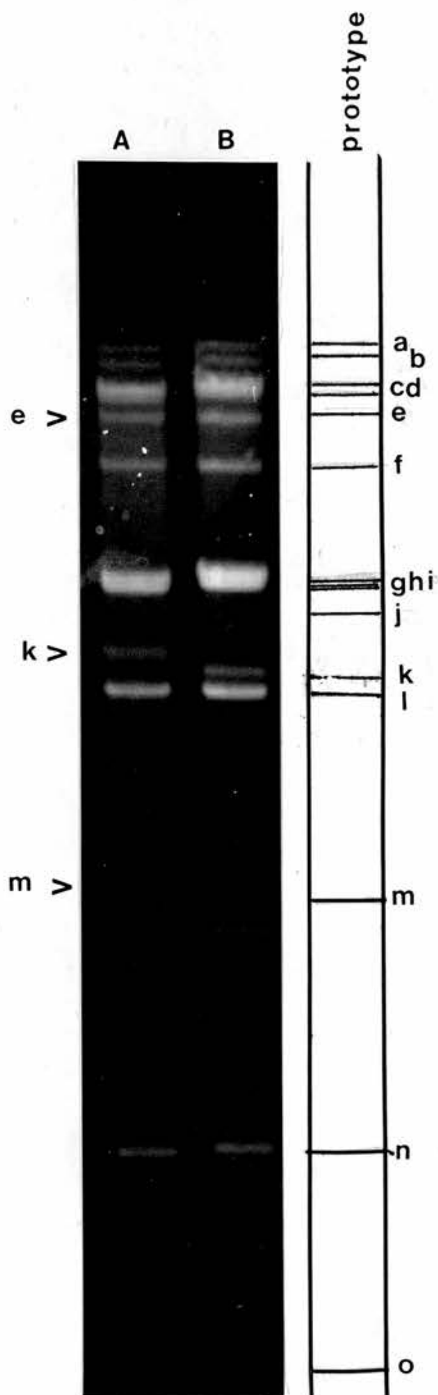


Figure 28

Agarose gel showing mobility differences obtained by digestion of HSV 2 DNA with BglII.

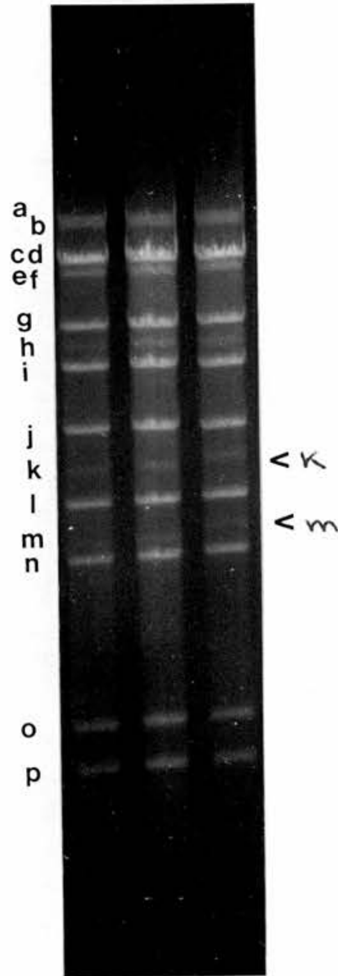


Figure 29

The map positions of HSV 1 DNA fragments exhibiting mobility variations during agarose gel electrophoresis.

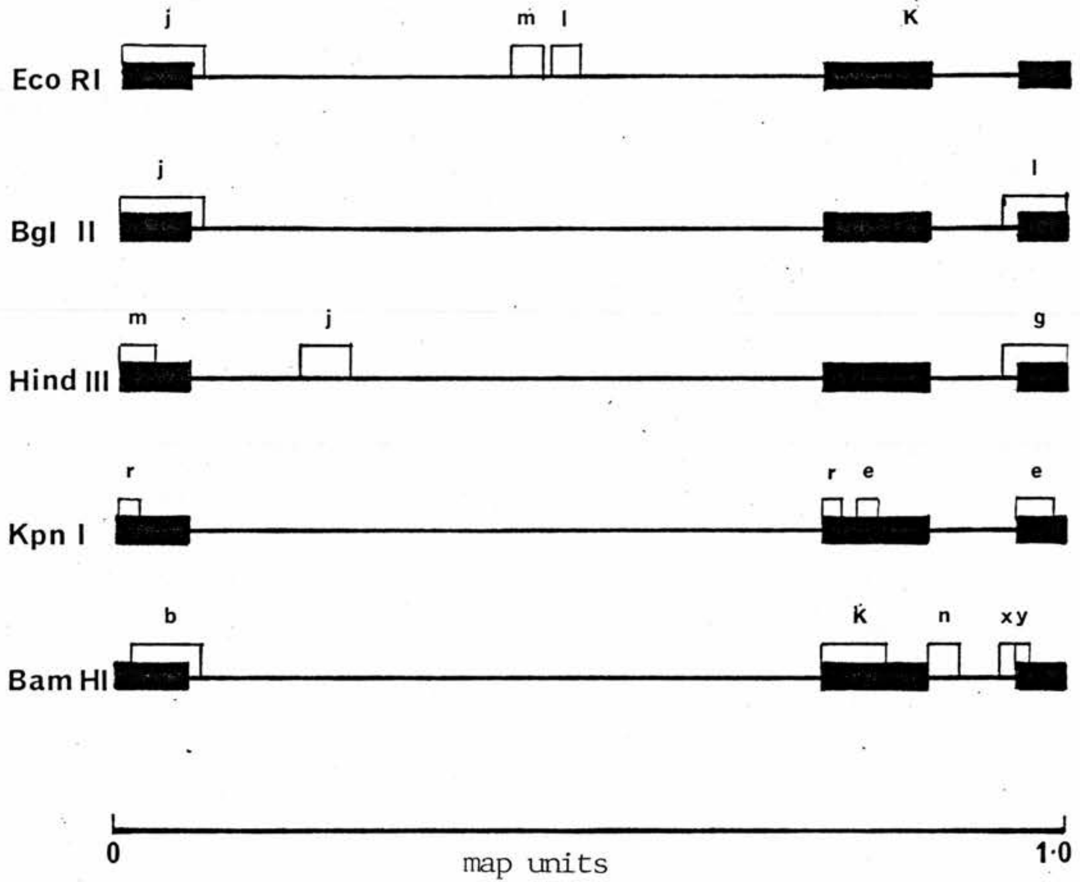
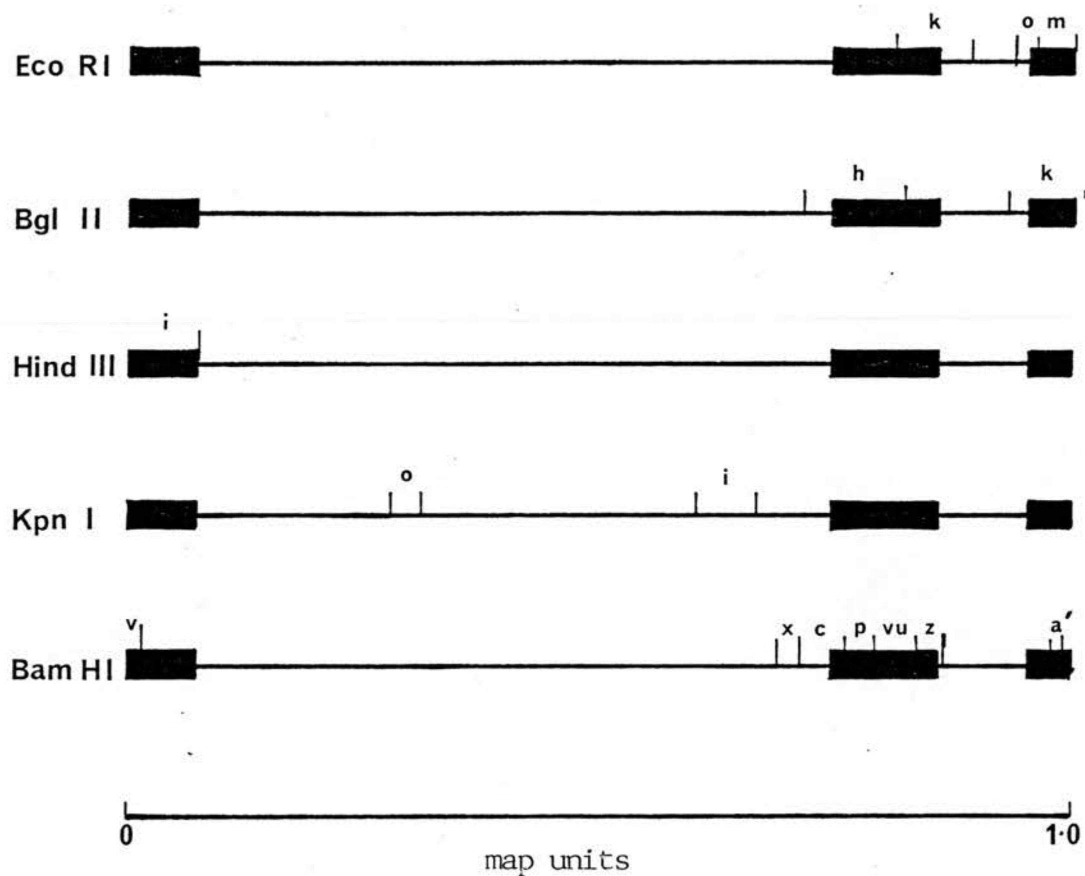


Figure 30

The map positions of HSV 2 DNA fragments exhibiting mobility variations during agarose gel electrophoresis.



fragments g (HSV 2) and k (HSV 1) not only varied in mobility but also in the number of bands generated.

Variations were also observed in the U_L region (e.g. HSV 1 EcoRI m and l, HindIII j; HSV 2, KpnI o and i) and the U_S region (e.g. BamHI n and x HSV 1) in a minimum of 2% of all isolates examined. However, several of these fragments were located adjacent to fragments in the joint or terminal repeat regions (e.g. HSV 2 BamHI c and x).

4. Mobility Variations in the Restriction Endonuclease Profiles of Clinical HSV Isolates

In order to determine the extent of variation in the electrophoretic mobility of restriction endonuclease fragments with site and/or time, selected groups of DNA preparations were digested to completion with the appropriate restriction endonucleases, then separated on agarose gels (as described in Methods section). The experimental design and specific groups were as follows:

(a) examination for variation of 8 HSV 1 viruses isolated concurrently from different anatomical sites of 3 patients (Table 10).

(b) examination for variation of 8 HSV 2 viruses isolated concurrently from different genital sites of 4 patients (Table 11).

Table 10

Clinical and restriction endonuclease data of 8 HSV 1 viruses isolated from different anatomical sites of 3 patients

Patient	Sex	DoB	Virus No	Site	DoI	Major RE profile	Mobility
D5876	F	28.11.45	1399	genitalia	22.03.77	S	BglII j
			1400	mouth	"		
M1693	M	08.06.47	1403	genitalia	22.03.77	S	BglII j
			1404	genitalia	"		EcoRI k
			1405	eye	"		BamHI k
			1406	mouth	"		
P2030	M	30.10.60	3088	genitalia	29.03.83	S	BamHI k
			3132	finger	"		

S = these isolates have identical major restriction enzyme profiles
DoI = date of isolation
DoB = date of birth
F = female
M = male

Table 11

Clinical and RE data of 4 patients from whom HSV 2 had been isolated concurrently at different genital sites

Patient	Sex	DoB	Virus No	Site	DoI	Major RE[a] profile	BamHI Mobility
E2692	F	26.01.53	10057	genitalia	20.03.82	ppxxw+	g,a'
			10058	cervix	"		
E2289	F	05.07.49	10137	genitalia	23.03.82	xppxw-	s,t,z
			10138	cervix	"		
E4557	F	18.01.58	30	vulva	16.06.83	xpxxw-	g,s
			31	cervix	"		
P4204	M	24.03.49	13226	genitalia	03.08.82	pppxw+	g,p,q
			13351	cervix	07.08.82		

M = male
F = female
DoB = date of Birth
DoI = date of Isolation
a = the order of digestion is EcoRI, BglII, HindIII, KpnI where p indicates prototype (as HG52), x indicate the following: for EcoRI, loss of f-g site; for BglII, extra site in c fragment; for HindIII, extra site in e fragment; for KpnI, loss of d-i site.
w = indicates presence (+) or absence (-) of BamHI w fragment

(c) A study of 14 sequential HSV 1 isolates from 7 patients (Fig 31) enabled examination of variation between the isolates of individuals over various time periods (Table 12).

Full clinical details of the patients in groups a, b and c are listed in Tables 10-12, however the REA of HSV 1 isolates are listed in the Appendix. RE maps for each endonuclease are provided for reference in the Appendix.

For each group (a, b and c) consistency of the overall restriction profile for each patient was observed with one exception, patient M3493 in Table 12. In this instance sequential genital HSV 1 isolates 4639 and 11849, obtained within a 5.9 year interval, were observed to differ in the deletion of KpnI m fragment. Variations due to the electrophoretic mobility of several fragments were noted for all isolates in groups a, b and c (as indicated in Tables 10-12), occurring mainly in the L-S junction or terminal fragments. In one instance (E1593, Table 10) the KpnI e fragment exhibited a decreased electrophoretic mobility between sequential isolates, to such an extent that the fragment mobility may mistakenly have been interpreted as a major variation.

5. Restriction Endonuclease Analysis of HSV DNA from Sexual Consorts

HSV DNA was prepared from 27 pairs (29 male, 25 female) of sexual partners with clearly defined clinical histories and whose serological status has been determined (see Methods). All 54 samples (42 HSV 2, 12 HSV 1) were initially examined 'blind' in an

Figure 31

Agarose gel showing KpnI digests of HSV 1 DNA from sequential genital isolates.

Tracks A, B - 2787, 6075
Tracks C, D - 4639, 11849
Tracks E, F - 3422, 8935
Track G - standard strain 17syn^{+}

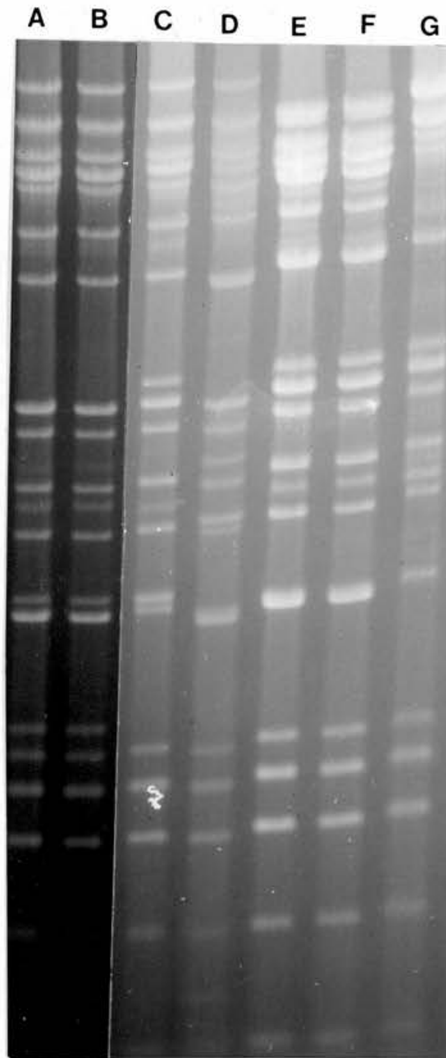


Table 12

Clinical and RE data of sequential genital HSV 1 isolates from 7 patients

Patient	Sex	DoB	Virus No	Site	DoI	Major RE profile	Mobility differences
D9830	F	30.07.63	961	External genitalia	05.03.81	S	EcoRI j
			10979	" "	29.04.82		
E234	F	16.08.64	16351	mouth	24.06.80	S	EcoRI j
			14277	genitalia	21.09.82		
E1491	F	22.07.61	2787	genitalia	26.05.81	S	KpnI k
			6075	genitalia	19.10.82		
E1593	F	04.07.63	3422	External genitalia	18.06.81	S	KpnI e
			8935	genitalia	04.02.82		KpnI k
E5303	F	23.11.54	5030	genitalia	23.12.83	S	Bam b
			7899	genitalia	06.04.84		KpnI r
							EcoRI k
E5309	F	11.03.60	5028	External genitalia	23.12.83	S	BglII o
			9018	genitalia	18.05.84		
M3493	M	18.03.57	4639	genitalia	25.05.78	Differs in	KpnI u
			11849	genitalia	27.08.84	KpnI m fragment	EcoRI k
						+ in 4639	
						- in 11849	

S = these isolates have identical major restriction enzyme profiles
+ = indicates presence of KpnI m fragment
- = indicates absence of KpnI m fragment

attempt to trace the transmission of HSV between sexual consorts. After obtaining the RE data, the clinical and serological background of each patient was examined to assess the significance of the results.

(a) HSV 1 isolates

The twelve HSV 1 isolates could be divided into seven separate groups on the basis of major variations as determined by the five restriction endonucleases EcoRI, BglII, HindIII, KpnI and BamHI (Fig 32). In five cases the virus profiles from paired sexual consorts were identical, however the remaining pair of isolates (1728, 2028) each had entirely different RE profiles. By reference to the serological and clinical data (Table 13), isolate 2028 was from a male patient who had suffered oral herpes 28 days before isolation of virus number 2028. In this instance the virus responsible for the oral lesion in the male patient may have been transmitted to the genitalia of the female consort during oro-genital sex. Isolation of virus 2028 from the male patient may therefore be caused by reactivation of a latent virus.

Three pairs of sexual consorts with primary serology had the same major RE profile as that of their respective consort (Table 14). This may be easily explained in two of the three cases where recent sexual partners other than the named consort were admitted. However in 1403/1399 no other sexual partners were admitted. Transmission of HSV may therefore have occurred by oral contact from a family member or unnamed individual.

Figure 32

Agarose gel showing BamHI digests of HSV 1 DNA from paired sexual consorts.

Track A - 1728
Tracks B, C - paired consorts 3079, 3088
Tracks D, E - paired consorts 4022, 4024

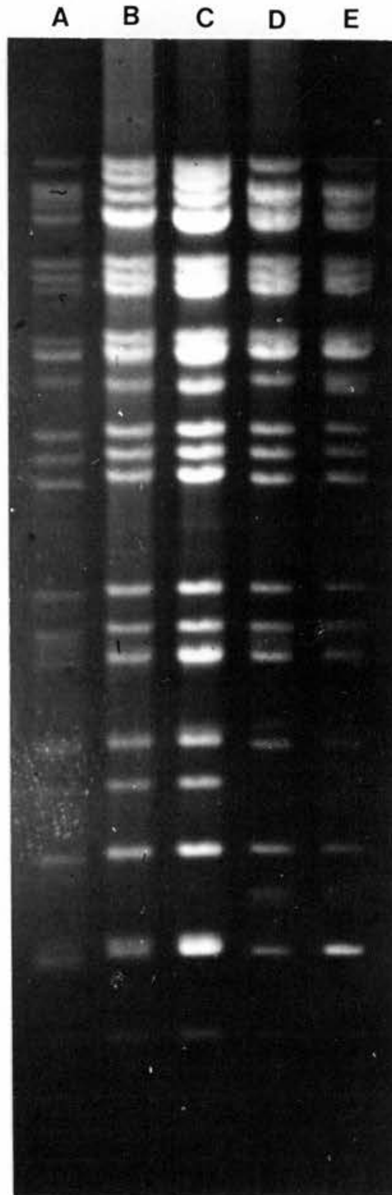


Table 13

Clinical, serological and RE data of paired sexual consorts from whom HSV 1 has been isolated

Virus no	Sex	Evidence of[a] other sexual relationships	Interval[b] (days)	Signs and symptoms	Serology	REA
1403	M	-	5	widespread lesions	P	S
1399	F	-	5	widespread lesions	P	
4024	M	-	10	penile herpes	P	S
4022	M	-	10	anal herpes	N-P	
8039	F	-	2	vulval herpes	P	S
8059	M	-	3	penile herpes	N-P	
1728	F	-	NA	vulval herpes	P	D
2028	M	-	NA	oral herpes 28 days before isolation	N-P	
3088	M	+	4	penile herpes	P	S
3079	F	-	5	vulval herpes	P	
3280	F	+	NA	vulval herpes	P	S
3349	M	-	NA	penile herpes	P	

M = male
F = female
a = + evidence of a recent sexual partner other than the named consort;
- no evidence of a recent sexual partner other than the named consort
b = time interval in days between last intercourse with named consort and virus isolation
P = primary infection; NP = non-primary infection
REA = restriction endonuclease analysis where S indicates the same major RE profile as the named consort and D indicates a different RE profile.

Table 14

Restriction endonuclease analysis of HSV 1 (DNA) from paired sexual consorts

Virus no	EcoRI	BglII	HindIII	KpnI	BamHI
1403	P	P	0-h	s-d	a2
1399				b	
				g	
				m2	
4024	P	P	o-h	s-d	a1
4022				b	
				g	
8059	P	P	P	s-d	a1
8039				b	
				g	
1728	P	P	P	s-d	a1
				m1	w-j'
2028	P	P	P	s-d	a1
3088	P	P	o-h	s-d	a2
3079				b	w-j'
				m1	
3280	P	P	P	s-d	a1
3349				b	w-j'
				g	d-h

P = prototype Re profile (as 17syn^{+})
- = fusion between the restriction fragments as stated for each endonuclease
b = new site in KpnI b fragment giving two new fragments of molecular weights 6.2 and 1.8Kbp
g = new site in KpnI g fragment giving two new fragments of molecular weights 5.2 and 1.8Kbp
m1 = new site in KpnI m fragment giving two new fragments of molecular weights 2.6 and 0.4Kbp
m2 = new site in KpnI m fragment giving two new fragments of molecular weights 1.8 and 1.1 Kbp
a1 = new site in BamHI a fragment giving two new fragments of molecular weights 7.2 and 0.8Kbp
a2 = new site in BamHI a fragment giving two new fragments of molecular weights 5.8 and 2.2Kbp

(b) HSV 2 isolates

Specific epidemiology proved to be very difficult with HSV 2 isolates due to the lack of major variations in their RE profiles (Table 15) (Figs 33, 34). In an attempt to clarify the relationships of some of the HSV 2 viruses, the clinical and serological data were examined in detail. In this instance 24 isolates were from patients who had either been involved in complex sexual relationships, or recorded a lengthy time interval between intercourse with the named consort and successful virus isolation (Tables 16, 17, 18). The remaining 18 isolates (9 pairs of sexual consorts) appeared to have no such complications (Table 18).

(i) Primary index cases with complications

Table 16 shows three primary index cases and their named consorts. In all three instances a sexual partner other than the named consort was admitted. Virus isolation in two of the three couples occurred at the same time or within a time interval of a few days. For the remaining couple (2437/9221) the female (9221) had an HSV isolation recorded in May 1984, although her regular partner had no isolation at this time. A previous specimen from the regular partner (2437) was isolated in March 1983 during a primary herpetic infection and could therefore be used for RE comparison. In all three pairs of named sexual consorts the RE profiles were found to be the same as that of their respective partner.

Figure 33

Agarose gel showing mobility differences in fragments g, p, q etc. on digestion of HSV 2 DNA with BamHI.

Track A - unrelated isolate
Tracks B, C - paired sexual consorts 13826, 9833
Tracks D, E - standard strain HG52

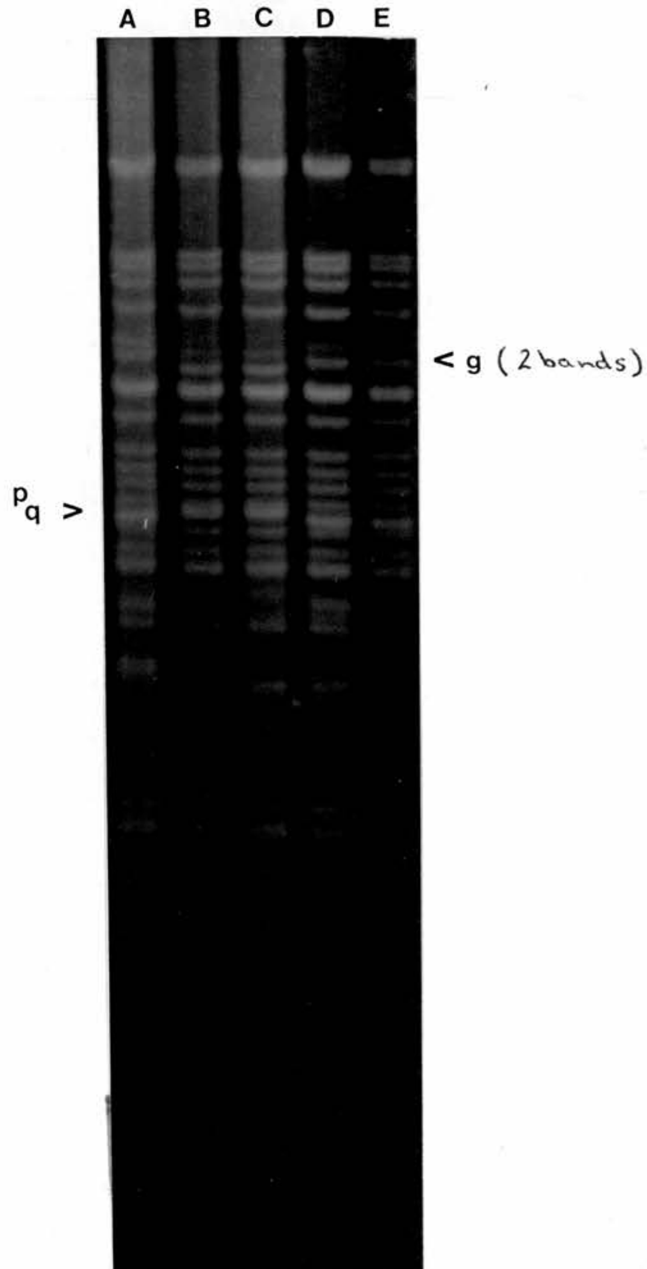


Figure 34

Agarose gel showing mobility differences in BamHI digests of HSV 2 DNA from paired sexual consorts (R1) (R2) and an unrelated isolate (U).

Fragment g appears as 2 bands, whereas fragments pq, st, vw and yz all appear as separate bands with variable mobilities.

Table 15

Variations among 42 HSV 2 (DNA) isolates from sexual consorts

Digestion pattern when restricted with: EcoRI BglII HindIII KpnI[a]	BamHI w[b]	Number of virus isolates
pppp	+	5
pppp	-	7
pppx	+	3
pppx	-	11
xppp	+	1
xppp	-	0
xppx	+	7
xppx	-	6
xpxx	+	0
xpxx	-	2

a = The order of digestion is, reading from left to right, EcoRI, BglII, HindIII, KpnI, where p indicates prototype (as HG52), x indicates the following: for EcoRI, loss of f-j site; for HindIII, extra site in e fragment; for KpnI, loss of d-i site.

b = BamHI digestion pattern indicates presence (+) or absence (-) of fragment w.

Table 16

Clinical, serological and RE data of HSV 2 isolates from paired sexual consorts with a lengthy time interval between last intercourse and viral isolation

Virus no	Sex	Evidence of other sexual relationships[a]	Date of last intercourse with casual consort	Date of last intercourse with named consort	Date of isolation	Signs and symptoms	Serology	REA[b]
13993	M	+	30.08.82	03.09.82	07.09.82	penile herpes	P	pppxw–
14086	F	-	-	"	09.09.82	vulval sores	N-P	
2437	M	+	NA	NA	04.03.83	penile herpes	P	xppxw⁻
9221	F	-	-	NA	24.05.84	vulval discomfort	I	
11249	M	+	--.06.84	20.07.84	03.08.84	penile lesions	P	xppxw⁻
11246	F	-	-	"	03.08.84	vulval herpes	N-P	

M = male
F = female
a = see footnote a of Table 13
b = see footnotes a and b of Table 15
NA = not available
P = primary infection
N-P = non-primary infection
I = initial infection

Table 17

Clinical and serological details of paired sexual consorts (13826 and 9833) with primary serology

Date	Male (13826)	Female (9833)
12.02.81	-	Negative serology, no virus isolation
--.11.81	Casual relationship with unnamed partner	-
17.12.81	Penile ulcer, primary serology, no virus isolation	-
09.02.82	-	Primary serology, no virus isolation
03.03.82	-	Primary serology, no virus isolation
11.03.82	-	Serology not available, virus isolation of 9833
12.03.82	No virus isolation	-
31.08.82	Virus isolation of 13826 from buttocks	-
08.09.82	-	No virus isolation

Table 18

Clinical, serological and RE data of HSV 2 isolated from paired sexual consorts with non-primary serology who had been involved in sexual relationships with unnamed individuals

Virus no	Sex	Evidence of[a] other sexual relationships	Interval (days)[b]	DoI	Signs and symptoms	Serology	REA[c]
1333	M	+	NA	22.01.83	penile herpes	N-P	$pppxw^+$
13468	F	-	3	12.08.82	vulval herpes	N-P	$pppxw^-$
2662	M	+	6	15.03.83	penile herpes	N-P	$pppxw^+$
3825	F	-	NA	29.04.83	vulval herpes	N-P	
11784	M	+	7	23.08.84	penile herpes	N-P	$ppppw^+$
3163	F	-	7	12.10.83	vulval sores	N-P	
13667	M	+	NA	14.02.80	penile herpes	N-P	$xppxw^+$
11259	F	-	3	06.08.84	vulval lesions	N-P	$ppppw^-$

M = male
F = female
N-P = non-primary infection
a = see footnote a on Table 13
b = time interval in days between last intercourse with named consort and virus isolation
c = see footnotes a and b on Table 15

Another couple (13826/9833; Table 17) both had evidence of primary infections. In this instance the samples used for RE analysis were isolated in 1982 after a recurrence. Both 13826 and 9833 were found to have the same major RE profile of ppppw^{+}.

(ii) Non-primary index cases with complications

The source patients of four pairs of sexual consorts had non-primary serology and admitted to being involved in other sexual relationships (Table 18). Two pairs of isolates (11784/3163 and 2662/3825) had identical RE profiles, however the isolates from the remaining two pairs of consorts each had a different RE profile from that of their named sexual partner. In these cases either virus transmission from an unnamed individual or reactivation of a latent virus may have taken place.

(iii) Uncomplicated non-primary infections in paired sexual consorts

Four pairs of sexual consorts, who on interview were recorded as having a stable relationship with the named sexual partner, were found to have non-primary infections (Table 19). All but three patients had clinical signs and symptoms of herpetic infections. Three of the four couples were found to have identical RE profiles on the basis of major variations. The remaining couple (3561/4989) had entirely different RE profiles from each other, despite having admitted to no other recent sexual relationships.

Table 19

Clinical, serological and RE data from paired sexual consorts with non-primary serology, who were in a stable relationship with the named sexual partner

Virus no	Sex	Time interval (days)[a]	Signs and symptoms	Serology	REA[b]
2884	M	21	penile herpes	N-P	pppxw⁻
2971	F	22	genital herpes	N-P	
3561	M	4	penile herpes	N-P	ppppw⁺
4898	F	2	vulval herpes	N-P	xpppw⁺
11010	M	NA	none	N-P	pppxw⁻
11640	F	NA	none	N-P	
15900	M	6	penile lesions	N-P	ppppw⁻
16492	F	NA	none	N-P	

a = see footnote a of Table 13
b = see footnotes a and b of Table 15
N-P = non-primary infection
M = male
F = female

(iv) Uncomplicated primary or initial index cases

Nine pairs of sexual consorts with primary or initial index cases were recorded as having a stable relationship with the named sexual partner (Table 20). One couple (7358/7495) were involved in homosexual practices. In all nine cases the RE profiles for each pair of sexual consorts was identical on the basis of major variations.

Two patients, 104 (male A) and 6801 (female A) were subsequently involved in a rather complex sexual relationship as shown in Fig 35. Couple A (104/6801) were proven to have the same virus strain by RE analysis of virus isolates in 1982. In 1983, 6801 was involved with another male (B) who was in contact both with his wife (B) and another female (B). Virus isolations from male B or wife B were unsuccessful, however isolate 3283 from female B was examined by RE analysis. In this instance 3283 was found to have the same profile as 104 and 6801 (ppppw$^-$).

6. Oro-genital HSV isolations

(a) Patients

Ten male patients (20-27 years) presenting with genital herpes or anal warts were found to have herpetic infections of the mouth. All patients had clinicl manifestations as characterised by tonsillitis (4), ulceration of the mucous membranes (3) and healing

Figure 35

Diagram of a complicated sexual relationship

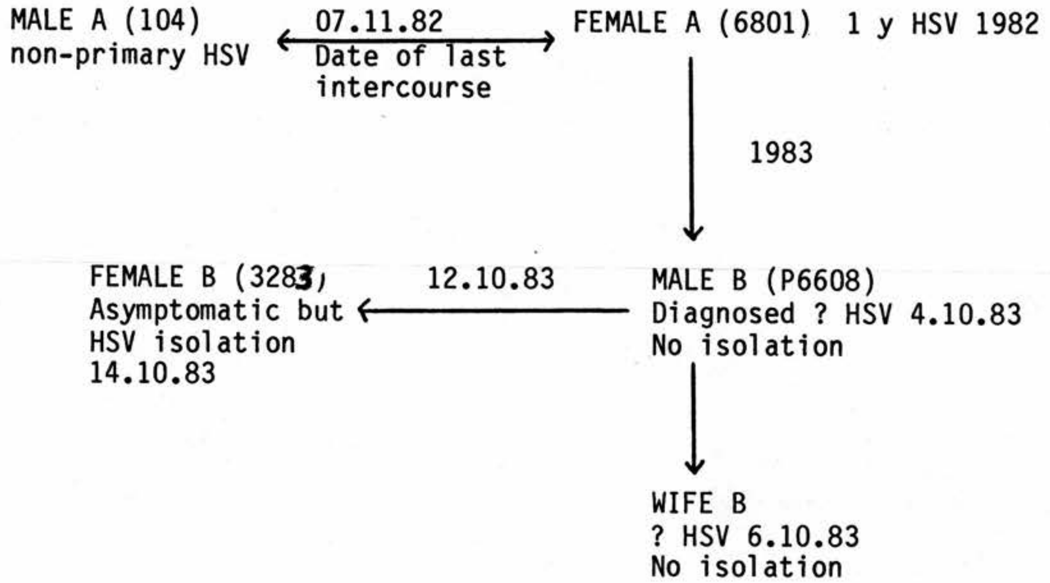


Table 20

Clinical, serological and RE data from paired sexual consorts with primary or initial index cases who were in a stable relationship with the named sexual partner

Virus no	Sex	Time interval (days)[a]	Signs and symptoms	Serology	REA[b]
9049	M	11	penile ulcers	P	xppxw+
9167	F	28	vulval lesions	N-P	
2884	M	21	penile herpes	I	pppxw-
2971	F	21	herpetic ulcer	N-P	
10080	M	7	penile herpes	P	ppppw-
11071	F	NA	none	NA	
12963	F	4	penile herpes	P	pppxw-
12929	M	4	vulval herpes	P	
6801	F	9	vulval herpes	P	ppppw-
104	M	19	penile blisters	N-P	
2597	M	5	penile lesions	P	xppxw+
3097	F	25	anal sores	N-P	
19	M	6	penile herpes	P	xpxxw-
30	F	7	vulval herpes	N-P	
6779	M	10	penile herpes	P	xppxw+
6593	F	7	vulval herpes	N-P	
7358	M	12	anal herpes	I	xppxw-
7495	M	14	oral herpes	N-P	

a = see footnote a of Table 13
b = see footnotes a and b of Table 15
M = male
F = female
P = primary infection
N-P = non-primary infection
I = initial infection
NA = not available

lesions on the lips (4). Other symptoms such as malaise, fever and gingivitis were also common. Nine patients yielded HSV from both oral and genital lesions while the tenth, a traced consort of a patient with genital herpes, yielded HSV from the throat. The isolates were all typed by immunofluorescence with ten (from five patients) found to be HSV 1 and the remaining nine to be HSV 2. All of the HSV 2 infections occurred from 1982 onwards with two of the patients admitting to homosexual practices.

(b) Serology

All five patients from whom HSV 1 had been isolated were found to be suffering primary infections (Table 21). Three of the five patients with HSV 2 infections were found to have a primary infection (Table 22), and of the remaining two patients one had an initial infection, whereas the other (P5612) was recorded as having had antibody to HSV 2 one year previously. No further clinical evidence was available in this instance to ascertain whether this was a first infection of the oral region.

(c) Restriction endonuclease analysis

HSV DNA was prepared from both the oral and genital specimens of each patient then analysed by the restriction endonucleases *EcoRI*, *BglII*, *HindIII*, *KpnI* and *BamHI*. On the basis of major RE variations all the HSV 1 genital isolates could be paired with that of the oral isolate from each respective patient. In each case a unique viral profile was obtained (Table 23). Examination of the HSV 2 isolates (Table 24) showed that isolates from three of five

Table 21

Clinical and serological data of 5 male heterosexual patients from whom HSV 1 had been isolated from both oral and genital sites

Patient	Age	Oral presentation	Genital presentation	Antibody status
P7829	20	Enlarged tonsils	Enlarged glands, herpetic ulcers	P
P6842	24	Herpes of the lips	Penile ulcers	P
P6032	32	Herpetic ulceration of the tongue	Penile ulcers	P
P2958	26	Herpetic ulceration of the hard palate	Preputial ulcers Enlarged glands	P
K9816	16	Ulceration of the lips	Penile rash Lymphadenopathy	P

P = primary infection

Table 22

Clinical and serological data for 5 patients from whom HSV 2 had been isolated from oral and genital sites

Patient	Sex	Age	Sexual practice	Oral presentation	Genital presentation	Antibody status
S592	M	20	Het	Herpetic ulceration of right tonsil	Penile ulcer lymphadenopathy	P
P9968	M	26	Hom	Herpetic ulceration of tonsils	Herpetic ulceration of anus and scrotum	I
P8863	M	27	Het	Herpetic lesions on tongue	Herpetic lesions on penis	P
P5612	M	22	Hom	Herpes of the upper lip	Anal warts	N-P
P4259	M	20	Het	Ulceration of tonsils	Penile ulcers Enlarged glands	P

M = male
Het = heterosexual
Hom = homosexual
P = primary infection
I = initial infection
N-P = non-primary infection

Table 23

Restriction endonuclease analysis of HSV 1 (DNA) isolated from patients with oral and genital lesions

Patient	EcoRI	BglII	HindIII	KpnI	BamHI
P7829	P	P	P	s-d b g p-v	a2
P6842	P	P	P	s-d b g	a1
P6302	P	P	P	s-d b g m1	w-j' d-h
P2958	a-k	P	o-h	s-d b	a1 j'-b'
K9816	P	P	o-h	s-d b g	a1

P = prototype RE profile (as 17syn+)
- = fusion between the restriction fragments as stated for each endonuclease
b = new site in KpnI b fragment giving two new fragments of molecular weights 6.2 and 1.8Kbp
g = new site in KpnI g fragment giving two new fragments of molecular weights 5.2 and 1.8Kbp
m1 = new site in KpnI m fragment giving two new fragments of molecular weights 2.6 and 0.4Kbp
a1 = new site in BamHI a fragment giving two new fragments of molecular weights 7.2 and 0.8Kbp
a2 = new site in BamHI a fragment giving two new fragments of molecular weights 5.8 and 2.2Kbp

Table 24

Restriction endonuclease analysis of HSV 2 (DNA) isolated from patients with oral and genital lesions

Patient	EcoRI	BglII	HindIII	KpnI	BamHIw
S592	P	P	P	P	(+)
P9968	P	P	P	d-i	(+)
P8863	P	P	P	d-i	(+)
P5612	f-j	P	P	d-i	(-)
P4259	P	P	P	d-i	(+)

P = prototype RE profile (as HG52)
\- = fusion between the restriction fragments as stated for each endonuclease
(+) = presence of BamHI w fragment
(-) = absence of BamHI w fragment

patients could not be distinguished solely on the basis of major variations.

(d) Comparison of the major RE profiles found in oro-genital HSV isolates with those from solely genital sites

Comparisons of the most common variable sites found in oro-genital and genital isolates are shown in Table 25a and b for HSV 1 and HSV 2 respectively. No notable differences were observed between the HSV 1 genital and oro-genital sites, however one site KpnI m (1.8/1.1) was not found in the oro-genital isolates. For HSV 2 isolates the frequency of KpnI d-i fusion was similar for both genital and non-genital isolates, however, the frequency of sites EcoRI f-j and BamHI w (1/1) in the genital isolates was more than double that of the oro-genital isolates. A larger sample of oro-genital isolates would be required for confirmation of these results.

7. HSV Isolations from Patients in London

Fifteen herpes simplex virus isolates were examined from persons living in the London area. Six of the HSV 1 isolates were from individuals (4 nurses B13-16 and two patients B17, 18) associated with a nosocomial outbreak in the intensive care unit of a London hospital. The remaining three HSV 1 and six HSV 2 isolates were random specimens from London patients.

Table 25

The frequency of variable RE sites in oro-genital and genital HSV isolates

(a) HSV 1 isolates

n	Site	BamHI a (7.2/0.8)	BamHI a (5.8/2.2)	BamHI d-h	BamHI w-j'	KpnI m (2.6/0.4)	KpnI m (1.8/1.1)	HindIII o-h
(5)	Oro-genital isolates	0.6	0.2	0.2	0.2	0.2	-	0.4
(44)	Genital isolates	0.5	0.27	0.16	0.27	0.29	0.2	0.32

(b) HSV 2 isolates

n	Site	EcoRI f-j	KpnI d-i	BamHI w(1/1)
(5)	Oro-genital isolates	0.2	0.8	0.2
(180)	Genital isolates	0.45	0.82	0.44

* The values in the table are x/n, where x is the number of isolates having the variable site and n is the total number of isolates in the group.

The DNA from all 15 isolates was examined by five restriction endonucleases EcoRI, BglII, HindIII, KpnI and BamHI. Tables 26 and 27 show the RE data for HSV 1 and HSV 2 isolates respectively. Three HSV 1 isolates, from one nurse (B16) and two patients (B17, 18) appeared to have the same RE profile, whereas the remaining three isolates (B13, 14, 15) had unique RE profiles and were not infected with the same HSV 1 strain as B16, 17 and 18 (Fig 36). The HSV 1 isolates from a random selection of individuals (B6, 9, 10) again showed a unique RE profile for each isolate.

By comparison, the DNA from six random HSV 2 isolates did not have unique RE profiles and therefore only one (B4) could be distinguished from the others on the basis of major variations (Table 27).

Consideration of the distribution of variable sites found in London isolates compared with those found in Edinburgh isolates is shown in Table 28. The HSV 1 isolates showed considerable variation between the Edinburgh (genital) and London (non-genital) isolates, with three of the seven most common variable sites not being found in the London isolates. In the remaining four sites only two (BamHI a (7.2/0.8) and d-h) showed a similar distribution between the London (non-genital) and the Edinburgh (genital) isolates. For HSV 2 (genital) isolates, only 2 of the 3 most common variable sites were found in the London isolates (Table 28b). As with the HSV 1 isolates, the distribution of variable sites in the HSV 2 isolates was found to differ between the London and the Edinburgh isolates, but may in part be due to the small number of isolates sampled.

Figure 36

Agarose gel showing BamHI digests of HSV 1 DNA isolated from patients and nurses in the London area. B16, 17, 18 are identical HSV 1 strains.

Track A, B10 - random isolate
Track B, B6 - random isolate
Track C, B18 - patient B, intensive care unit
Track D, B17 - patient A, intensive care unit
Track E, B16 - nurse B, intensive care unit
Track F, B15 - nurse A, intensive care unit

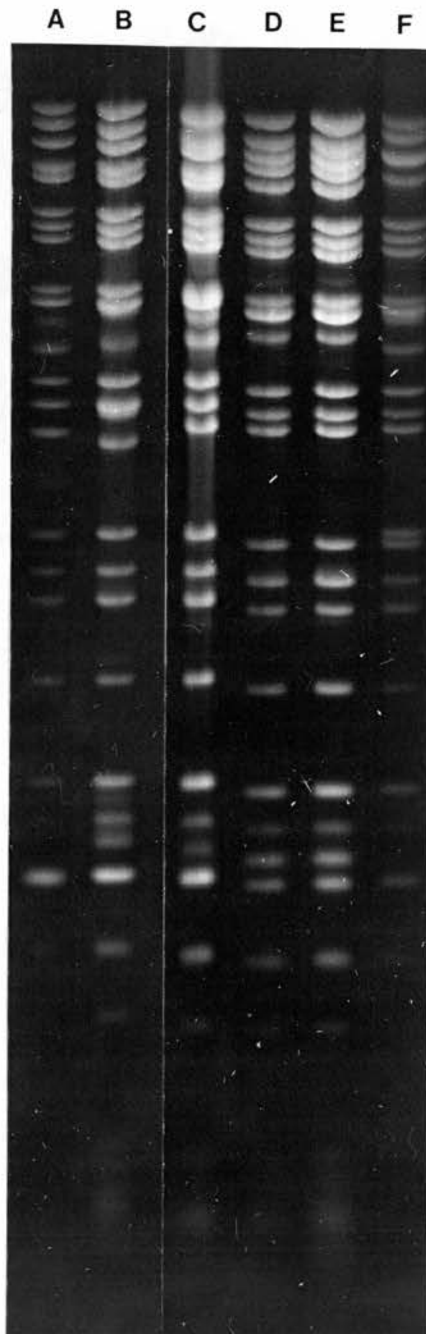


Table 26

Restriction enconuclease analysis of HSV 1 (DNA) isolated from nurses, patients and other individuals in the London area

Virus no	EcoRI	BglII	HindIII	KpnI	BamHI w
B6	P	P	o-h	s-d b g	a1 j'-b'
B9	P	P	P	s-d b g	a1
B10	P	P	o-h m-l	s-d b g p-v	
B13	P	P	o-h	s-d b g	d-h
B14	P	P	m-n	s-d b g	 w-j'
B15	P	P	o-h	s-d b g	 w-j'-b'
B16	P	P	P	s-d m2	a1 j'-b'
B17	P	P	P	s-d m2	a1 j'-b'
B18	P	P	P	s-d m2	a1 j'-b'

P = prototype RE profile (as 17syn+)
- = fusion between the restriction fragments as stated for each endonuclease
b = new site in KpnI b fragment giving two new fragments of molecular weights 6.2 and 1.8Kbp
g = new site in KpnI g fragment giving two new fragments of molecular weights 5.2 and 1.8Kbp
m2 = new site in KpnI m fragment giving two new fragments of molecular weights 2.6 and 0.4Kbp
a1 = new site in BamHI a fragment giving two new fragments of molecular weight 7.2 and 0.8 Kbp

Table 27

Restriction endonuclease analysis of HSV 2 (DNA) isolated from London inhabitants

Virus no	EcoRI	BglII	HindIII	KpnI	BamHI w
B1	P	P	P	d-i	(+)
B2	P	P	P	d-i	(+)
B3	P	P	P	d-i	(+)
B4	f-j	P	P	d-i	(+)
B5	P	P	P	d-i	(+)
B8	P	P	P	d-i	(+)

P = prototype RE profile (as in HG52)
- = fusion between the restriction fragments as stated for each endonuclease
(+) = presence of BamHI w fragment

Table 28

The frequency of variable RE sites found in London and Edinburgh HSV isolates

(a) HSV 1 isolates

n	Site	BamHI a (7.2/0.8)	BamHI a (5.8/2.2)	BamHI d-h	BamHI w-j'	KpnI m (2.6/0.4)	KpnI m (1.8/1.1)	HindIII o-h
(7)	London isolates (non genital)	0.43	-	0.14	-	-	0.14	0.57
(44)	Edinburgh isolates (genital)	0.5	0.27	0.16	0.27	0.29	0.2	0.32

(b) HSV 2 isolates (genital)

n	Site	EcoRI f-j	KpnI d-i	BamHI w(1/1)
(6)	London isolates	0.17	1.0	-
(180)	Edinburgh isolates	0.45	0.82	0.44

* The values in the table are x/n, where x is the number of isolates having the variable site and n is the total number of isolates in the group.

8. HSV in Renal Transplant Recipients

(i) Patients

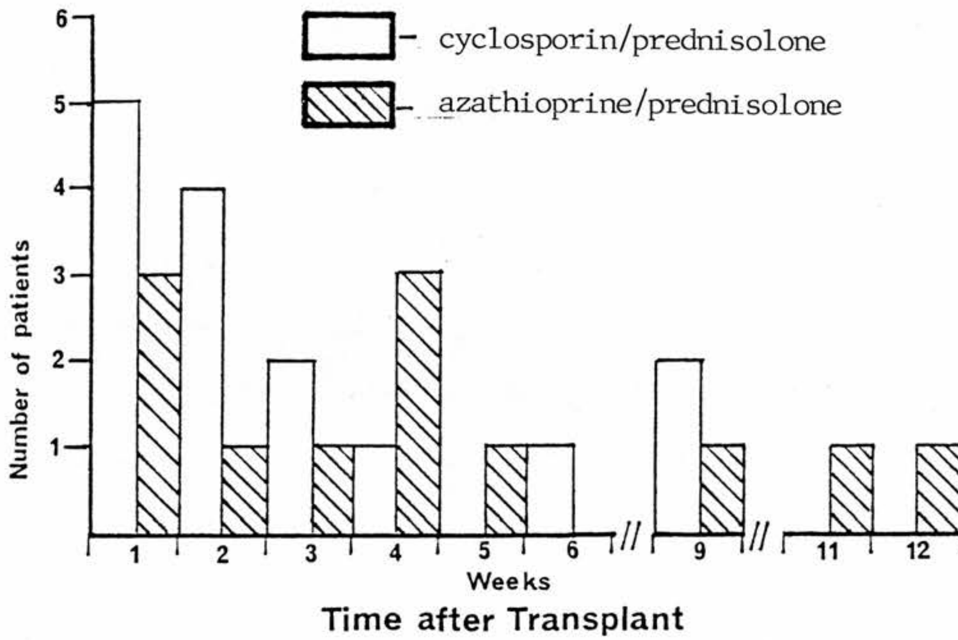
During the study of 50 renal transplant recipients HSV was isolated from the throat swabs of 27 patients (13 male, 14 female) with an age range of 31-60 years (average age 47 (male), 35.5 (female)). Fifteen of the 27 patients (56%) received a combination of cyclosporine (CsA) and prednisolone (P) with the remaining 12 (44%) were treated with azothioprine (A) and prednisolone.

Figure 37 illustrates the time interval between receiving a transplant and the first isolation of HSV from the throat swabs of patients receiving CsA/P compared to that of the azothioprine/prednisolone (A/P) treated patients. Individuals on CsA/P therapy were found to shed the first virus isolate within a 9 week period, with the majority of patients shedding virus between 1-4 weeks. However, those patients treated with A/P had a first virus isolate as late as 12 weeks after treatment.

All 27 patients were sampled at regular intervals for a minimum of 6 weeks after transplant, although those patients on A/P were followed for a longer period. During this period 8 patients were defined as shedding HSV, since a number of sequential HSV isolates had been recovered from throat swabs over a period of 4 weeks or more.

Figure 37

The time interval between transplantation and first isolation of HSV from renal transplant recipients receiving two types of immunosuppressive therapy.



(ii) Restriction endonuclease analysis

Forty four HSV 1 samples isolated from throat swabs of 16 (9 male, 7 female) renal transplant recipients were examined by RE analysis using EcoRI, BglII, HindIII, KpnI and BamHI restriction enzymes. The pre-transplant antibody status was determined in 13 of the patients (Table 29); the remaining three patients had no serum available for testing.

(a) Possible Transmission of Virus Infection in an Open Ward

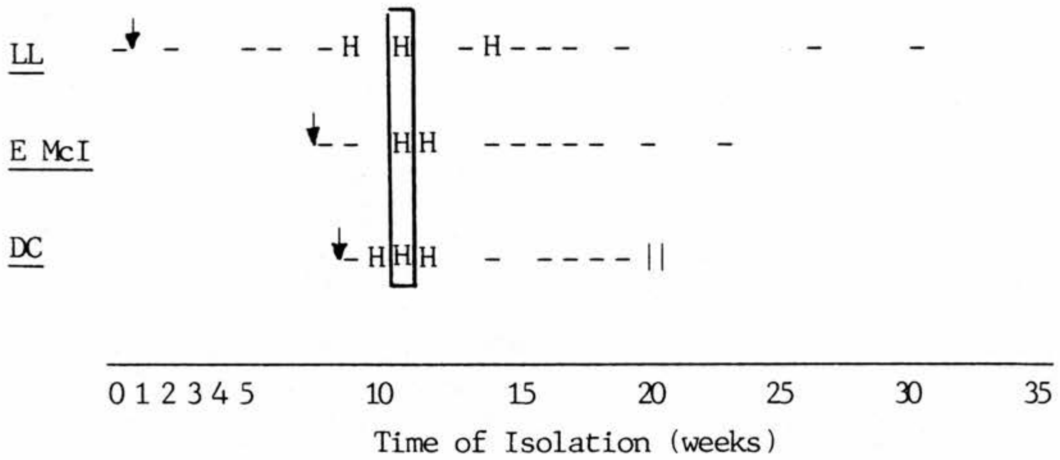
Three of the 16 RTRs (Fig 38) all yielded HSV during treatment in an open hospital ward. Patient LL, from whom HSV had been isolated a few weeks before transplant, was treated in an open ward beside patients EMcI and DC. Several weeks later all three patients yielded HSV from throat swabs (see Fig 38). By examination of the RE profiles of the HSV DNA from each patient (Fig 39), it was established that all three individuals had been suffering from a unique HSV 1 strain and therefore no transmission of HSV 1 had occurred within the ward.

(b) The Possible Transmission of HSV from Infected Kidneys to Recipients

Ten individuals received a kidney from 5 cadavers, however only two receiving kidneys from the same cadaver suffered HSV infections after transplantation (Fig 40). Both subsequently had a nephrectomy after three months. In order to determine whether both patients had been infected with an identical strain of HSV, the RE profiles of

Figure 38

HSV isolations from renal transplant recipients who had been treated in an open ward



↓ Time of transplant

H HSV isolation

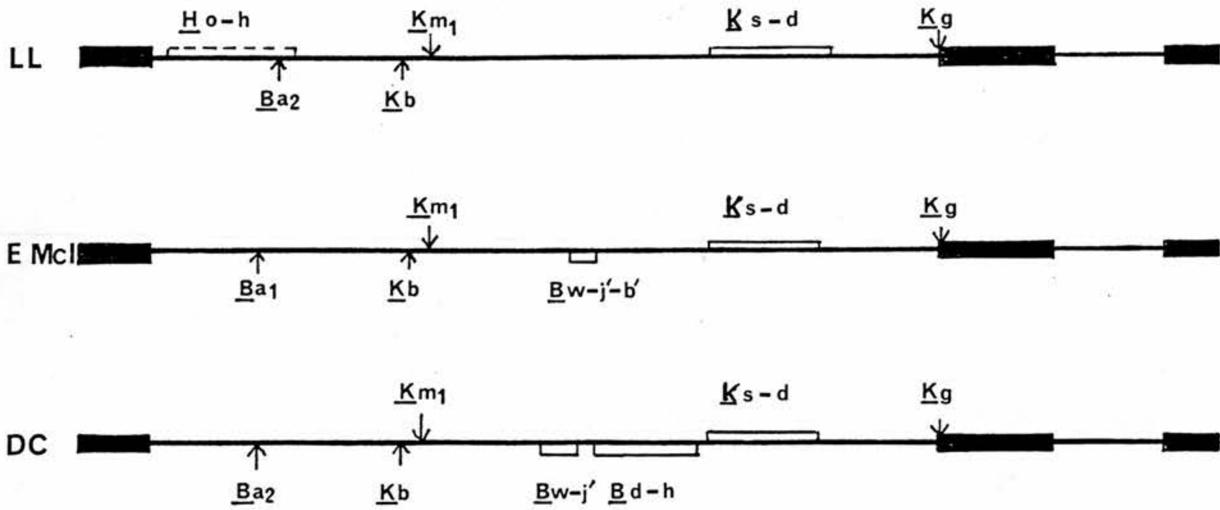
\- Negative isolation

|| Nephrectomy

▯ Possible transmission period

Figure 39

Restriction endonuclease profiles of HSV 1 DNA isolated from renal transplant recipients who had been treated in an open ward.



↓ new site

⌐ ¬ fusion

B = BamHI

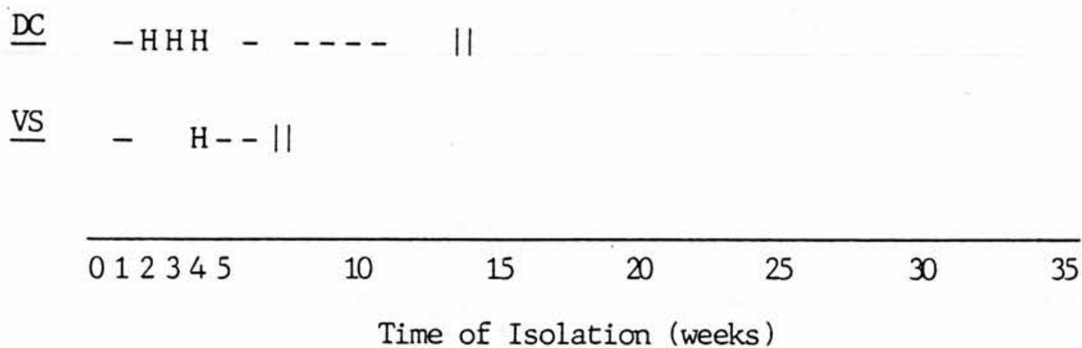
K = KpnI

H = HindIII

New sites and fusions are as described in Table 14.

Figure 40

HSV isolations of 2 renal transplant recipients who had received a transplant from the same cadaver



H HSV isolation

– Negative isolation

|| Nephrectomy

Table 29

Clinical and serological data of 16 renal transplant recipients examined by restriction endonuclease analysis

Patient	Age	Sex	Ab at time of transplant	Period sampled (months)	Number of throat swabs	Number of HSV isolated	Number examined by RE
JR	56	M	+	14	25	9	6
HMcK	57	M	NA	13	19	2	2
MCL	20	F	NA	12	20	1	1
MC	33	F	+	11	17	2	2
WS	41	M	+	10	21	2	2
TMcG	31	F	+	10	15	1	1
LL	31	M	+	9	17	3	3
IG	48	F	+	9	13	5	4
EMcI	42	F	+	6	15	2	2
DL	60	M	+	4	10	5	5
JB	45	M	+	2.5	8	4	2
JC	15	M	+	2.5	8	4	4
EB	16	F	+	2.5	11	6	4
DC	56	M	+	3*	9	3	3
VS	20	F	+	1.5*	4	1	1
AC	51	M	NA	3*	8	3	2**

RE = restriction endonucleases
* = time of nephrectomy
** = post nephrectomy
NA = not available
\+ = antibody to HSV detected by Complement Fixation test

the HSV 1 DNA from each patient was examined (Fig 41). In this instance a different RE profile was obtained for each patient, therefore no common HSV 1 strain was responsible for infection. In both cases HSV was isolated solely from throat swabs and antibody was present before the transplantation. No information regarding the HSV antibody status of the donor was available.

(c) RE Analysis of HSV Isolates from Three Patients Shedding Virus

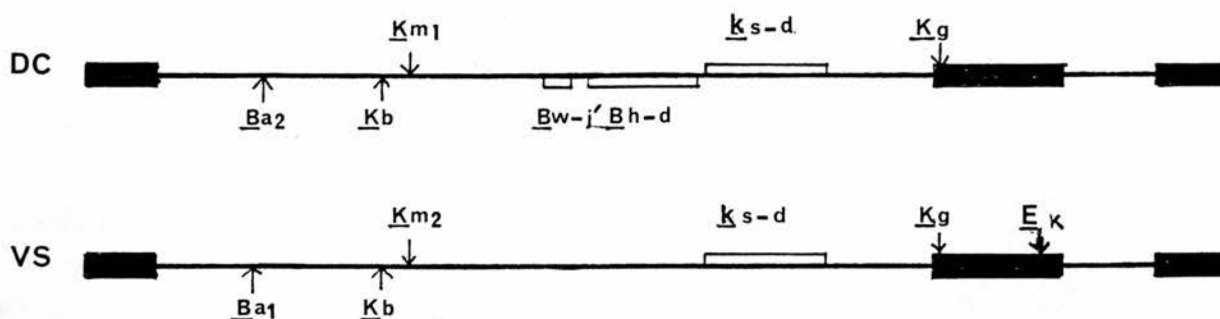
HSV DNA from three patients were examined by RE analysis. Patient JR (Fig 42) was sampled over a 14 month period and found to shed virus over six months. Patient IG was sampled over a nine month period, shedding virus over six weeks, as did patient DL who was sampled over four months. All positive HSV isolations were examined by RE analysis with the exception of one, which was not available (patient IG). The RE profiles of six samples from JR and 5 samples from DL showed no major variations in sequential isolates from each patient. Examination of four isolates from IG showed a major variation in the RE profile of one isolate, occurring in the unique long segment of the genome (KpnI m fragment) (see Appendix). Comparison of the RE profiles from all three patients (Fig 43) showed that each had a different HSV 1 strain.

(d) RE Profiles of the sixteen renal transplant recipients

Examination of sequential isolates from all 16 RTRs showed that each patient had a unique strain of HSV 1 as determined by the RE profiles (see Appendix), and with one exception (IG) demonstrated no major variations in sequential virus isolations from the same

Figure 41

Restriction endonuclease profiles of HSV 1 DNA from two renal transplant recipients who had received a transplant from the same cadaver.



new site

fusion

B = BamHI

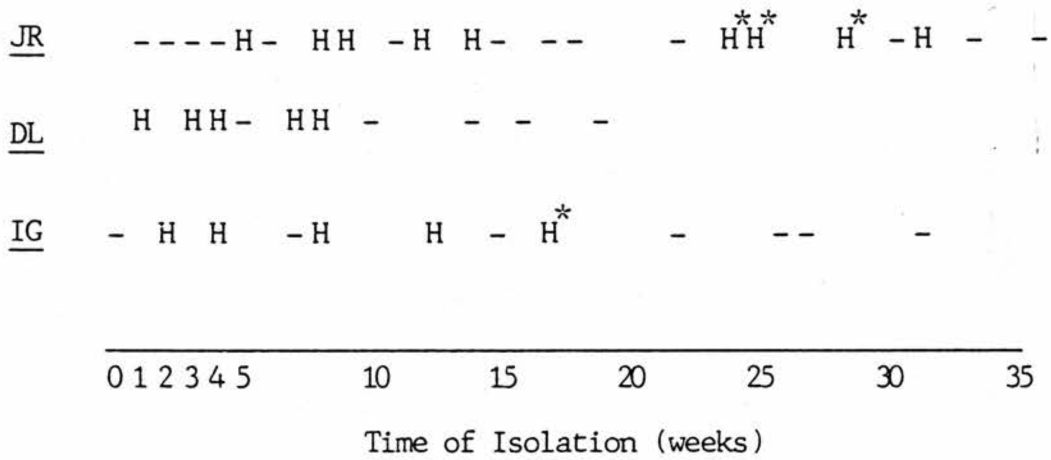
E = EcoRI

K = KpnI

New sites and fusions are as described in Table 14.

Figure 42

Patients shedding HSV over a period of six weeks or more



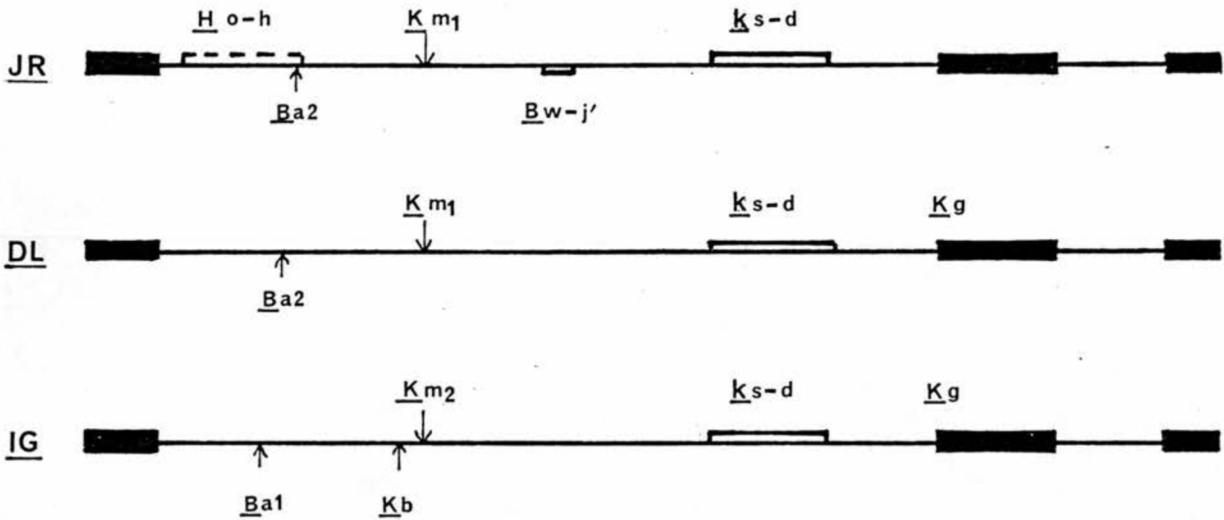
* Not examined

H HSV isolation

\- Negative isolation

Figure 43

Restriction endonuclease profiles of 3 renal transplant recipients who shed HSV over a period of 6 weeks or more.



↓ new site

fusion

B = BamHI

K = KpnI

H = HindIII

New sites and fusions are as described in Table 14.

individual. Consideration of the variability of RE sites for patients receiving A/P therapy compared to CsA/P therapy demonstrated no significant difference either in the number or distribution of RE sites (data in Appendix).

(e) Comparison of the Frequency of the Most Variable RE Sites in Non-genital (RTR) and Genital Isolates

Table 30 illustrates the frequency distribution of non-genital HSV isolates compared with genital HSV isolates from the Edinburgh area. Only three (BamHI a (5.8/2.2), BamHI d-h, KpnI m (1.8/1.1)) of the seven most variable sites were found to have the same frequency of distribution in both genital and non-genital isolates. The most notable difference was found in KpnI m (2.6/0.4) site where the frequency of the non-genital isolates was almost double that of the genital isolates (see Table 30).

9. Polyacrylamide Gel Electrophoresis (PAGE)

Polyacrylamide gel electrophoresis of selected HSV DNA samples was carried out then stained with an ultrasensitive silver stain as described in the Materials and Methods. Using standard strain DNA (HG52 and 17syn$^+$) the reproducibility of the technique was established, then by using different preparations of the standard strain DNA (Fig 44) any variable fragments were noted. Small molecular weight fragments were easily detected with good resolution particularly in the regions of the genome ranging from 2-0.5Kbp. Fragments of molecular weight standard Ø x 174RF/HaeIII were extracted from agarose gels, purified (see Materials and Methods)

Figure 44

Polyacrylamide gel electrophoresis of standard strains (17 (HSV 1) and HG52 (HSV 2)) and sequential HSV 1 isolates from Renal Transplant Recipients.

Tracks A, ØX174 RF/ HaeIII ; B, 17 (BamHI) ; C, 17 preparation 2 (BamHI) ; D, repeat of track B ; E, HG52 (BglII) ; F, HG52 (BamHI) ; G,H, patient JR 138,98 (BamHI) ; I,J, patient LL 613,558 (BamHI) ;K-N, patient DL 850,822,791,772 (BamHI).

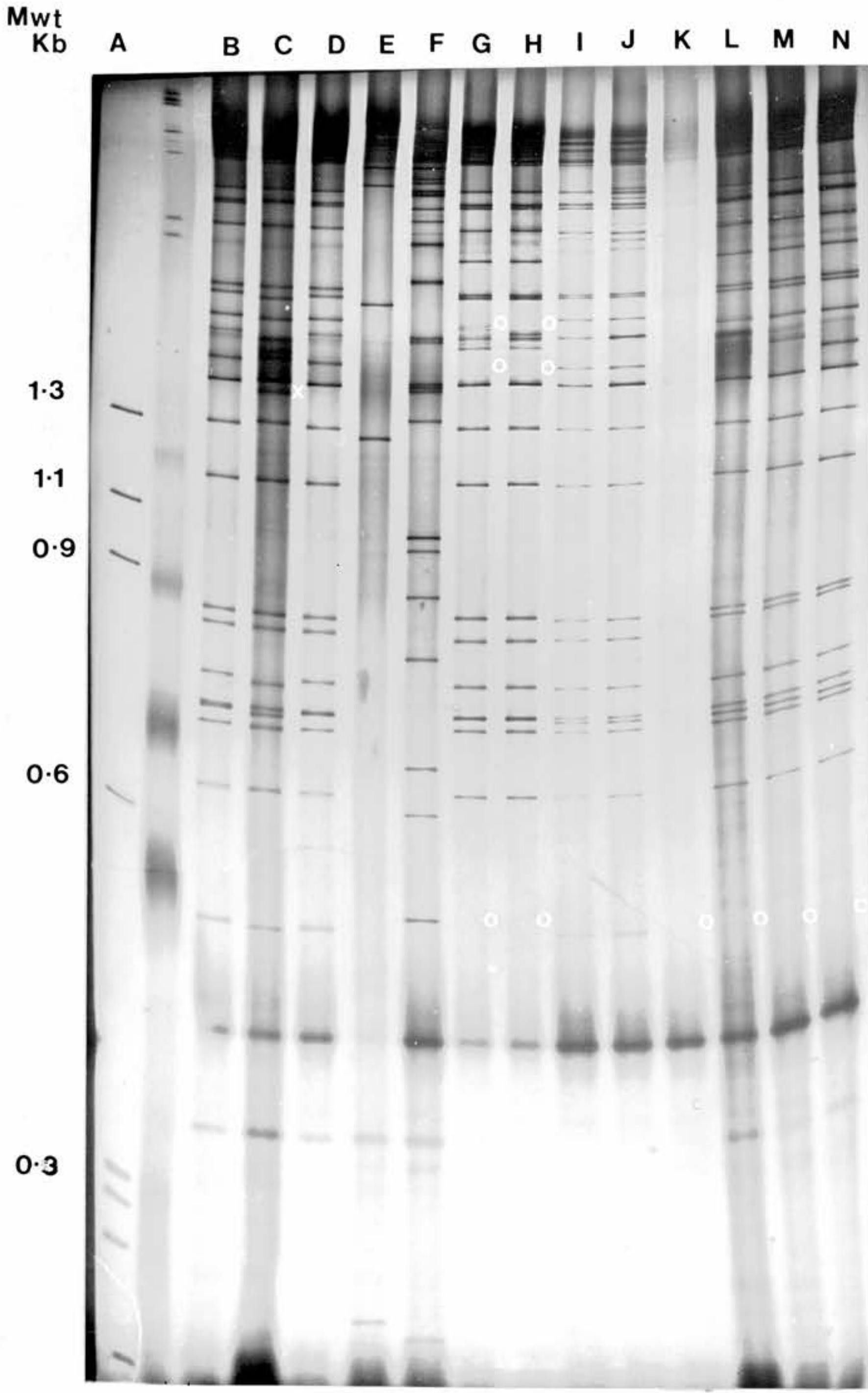


Table 30

The frequency of variable RE sites in non-genital (renal transplant throat swabs) and genital HSV 1 isolates*

n	Site	BamHI a (7.2/0.8)	BamHI a (5.8/2.2)	BamHI d-h	BamHI w-j'	KpnI m (2.6/0.4)	KpnI m (1.8/1.1)	HindIII o-h
(16)	Non-genital (RTR)	0.62	0.25	0.12	0.12	0.50	0.25	0.25
(44)	Genital	0.50	0.27	0.16	0.27	0.29	0.21	0.32

* The values in the table are x/n, where x is the number of isolates having the variable site, and n is the number of isolates in each group.

then run on a polyacrylamide gel. In each case the molecular weight fragment ran to approximately the same distance as that shown by the respective fragment of Ø x RF174 on the polyacrylamide gel (data not shown.

(a) Variations in RE profiles from sequential oral and genital HSV isolates

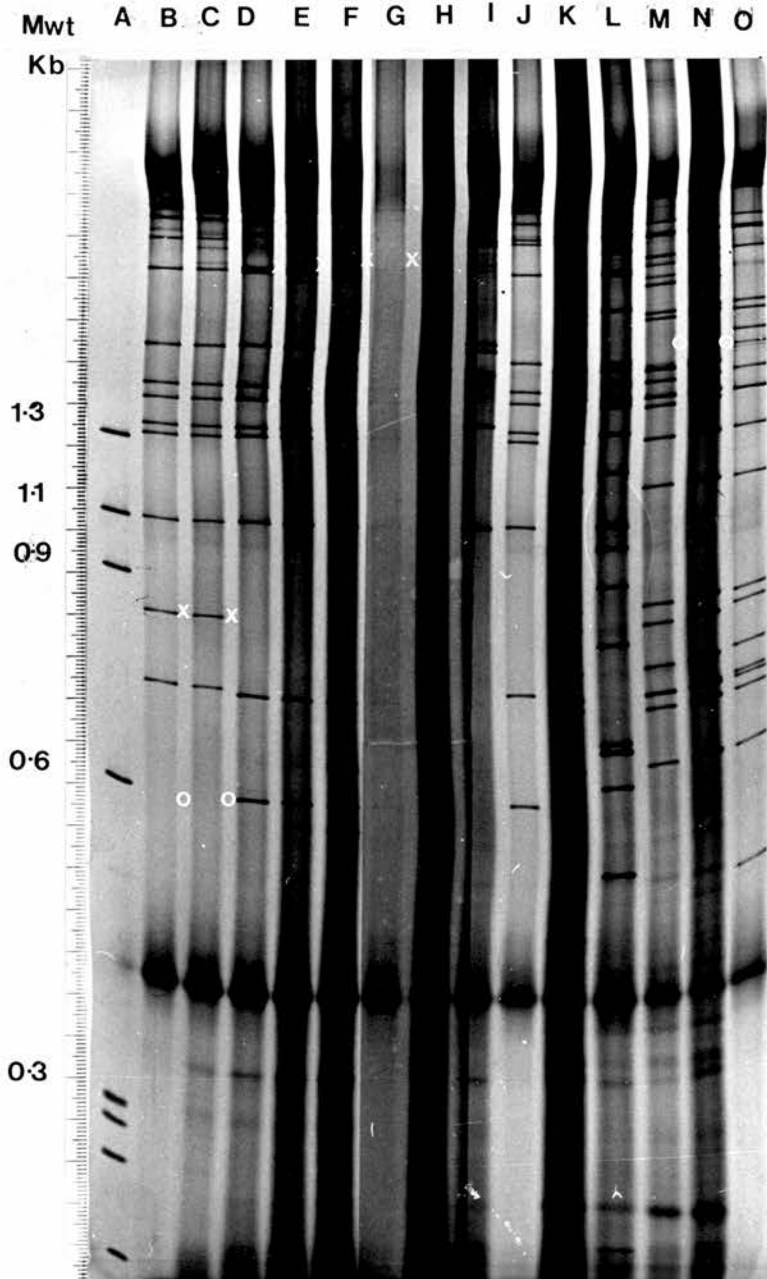
(i) Eight HSV 1 samples isolated sequentially from throat swabs of three renal transplant recipients JR, DL and LL, were digested with either KpnI or BamHI and examined by polyacrylamide gel electrophoresis as described in the Materials and Methods. The results for electrophoresis are shown in Figs 44 and 45, however the clinical and serological data have been described previously (see Table 29). Figure 45 shows consistency of mobility and number of RE fragments for sequential isolates from patients JR and DL when each sample was digested with KpnI. Examination of the same samples with BamHI (Fig 44) revealed very small mobility differences for patient DL (Tracks K-N) in fragments of ~ 1.35-1.4Kb. A similar difference was also noted between the sequential isolates from patient JR. However, examination of sequential isolates from a third patient, LL, found no variation at this position. Several differences in a number of high molecular weight fragments were noted between Tracks I and J.

(ii) Sequential HSV 1 samples isolated from the genitalia of two individuals were digested with BamHI and examined by PAGE as shown in Tracks LMN of Fig 45. Samples 8935 and 3422

Figure 45

Polyacrylamide gel showing : KpnI digests of sequential HSV 1 isolates from Renal Transplant Recipients (B-G) ; KpnI and BamHI digests of sequential genital isolates (H-N) and standard HSV 1 (17) DNA.

Tracks A, ØX174RF / HaeIII ; B,C, patient JR 98, 138 ; D-G, patient DL 772,791,822,850 ; H, 961 (KpnI) ; I, HSV 2 (KpnI) ; J, 17 (KpnI) ; L, HSV 2 (BamHI) ; K, 961 (BamHI) ; M, 8935 (BamHI) ; N, 3422 (BamHI) ; 0, 17 (BamHI).



(paired sequential isolates) had identical RE profiles that revealed no apparent mobility differences. The RE profiles of 8935 and 3422 differed substantially from that of 961 (Track L) an unrelated isolate.

(b) Variations in the RE profiles of HSV from paired sexual consorts

(i) Eight HSV 1 and eight HSV 2 samples isolated from eight pairs of known sexual consorts, were digested with BamHI then examined by PAGE. (The clinical and serological data for these samples have already been described in Tables 12 and 13). By reference to the standard strain 17syn+, fragment deletions and extra fragments were denoted as (O) or (X) for HSV 1 isolates, as shown in Figure 46. Tracks B and C revealed identical RE profiles with no mobility differences observed. Unfortunately tracks B and D were very faint and cannot be seen clearly in the figure provided. Tracks F and G exhibited similar RE profiles, however trace bands in track F are attributed to small amounts of host DNA in the sample. Variations between isolates H and I were found in the form of an extra band (in Track I) of approximately 1.5Kb.

(ii) Similar studies with HSV 2 isolates from paired sexual consorts revealed mobility variations between paired isolates as indicated by (**O**) on Figure 47. Several fragments were observed to differ between isolates D and E (at approximately 1.8Kb) that could not be attributed to host contamination or an incomplete digestion pattern. Examination of tracks F and G also revealed differences in mobility and number of restriction fragments, despite the small

Figure 46

Polyacrylamide gel showing BamHI digests of HSV 1 DNA from paired sexual consorts.

Tracks A, ØX174RF / HaeIII ; B, 4024 ; C, 4022 ; D, 3088 ; E, 3079 ; F, 1728 ; G, 2028 ; H, 8039 ; I, 8059 ; J, λ / HindIII .

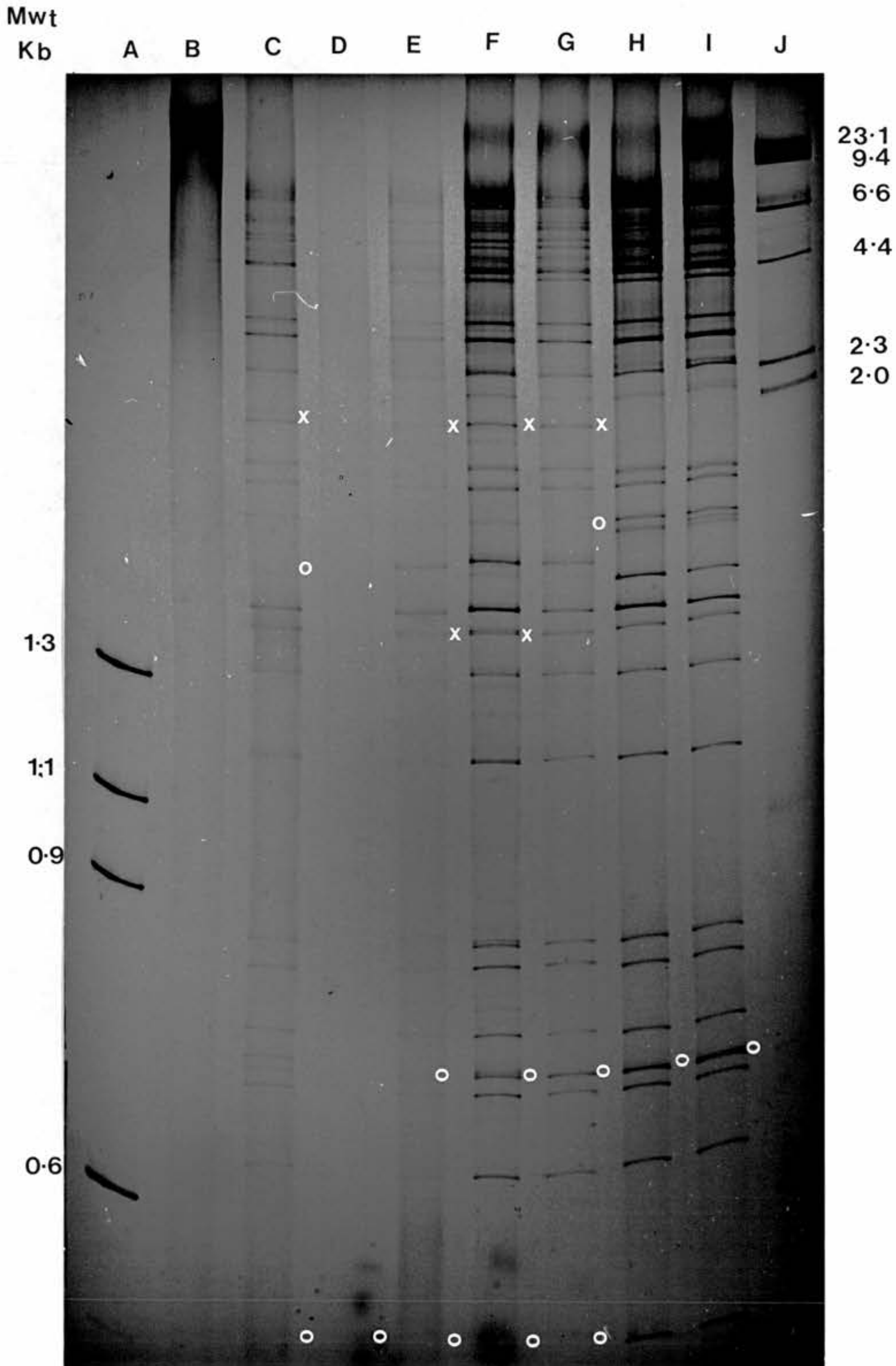
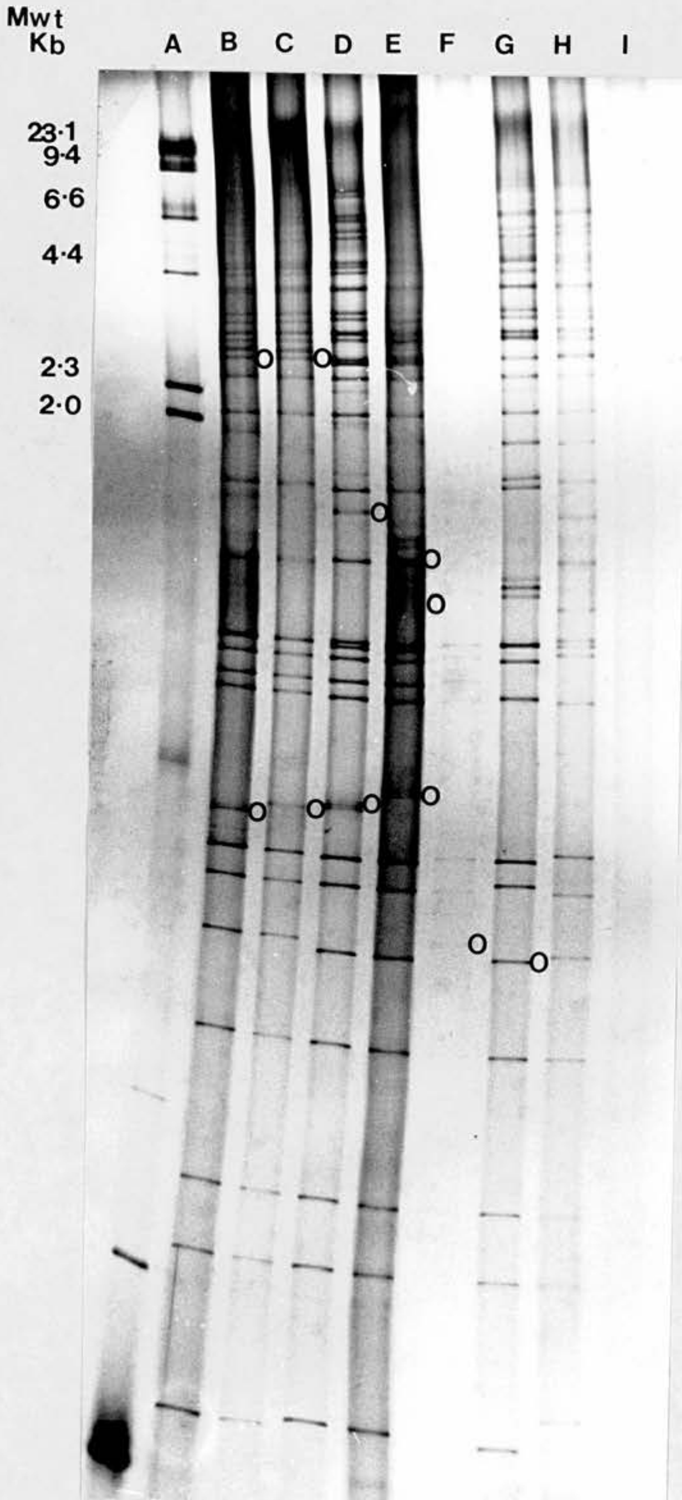


Figure 47

Polyacrylamide gel showing BamHI digests of HSV 2 DNA from paired sexual consorts.

Tracks A, λ/ HindIII ; B, 19 ; C, 30 ; D, 11010 ; E, 11640 ; F, 11080 ; G, 11071 ; H, 1333 ; I, insufficient sample.



quantity of DNA in Track F. No comparison of Tracks H and I could be clearly obtained due to insufficient amounts of sample (Track I) available.

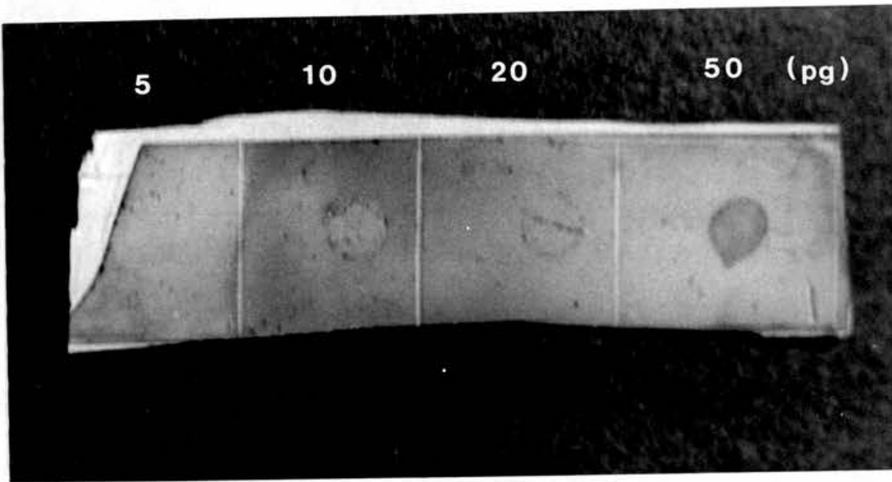
10. Colorimetric Detection of Biotin-labelled Nucleic Acids

(a) Sensitivity

Nitrocellulose filters were spotted with biotinylated standard DNA, baked, then subjected to the colorimetric procedures described in the Materials and Methods section. After one hour a blue-coloured precipitate formed in the solution, attaching on to the nitrocellulose. After 4.5 hours incubation only one dot (50pg) of biotinylated DNA developed. This procedure was repeated with fresh, filtered buffers and the same result was obtained. Two subsequent colorimetric solutions obtained from BRL produced the same results. A final dye solution provided by BRL produced a slightly better result with the 50pg dot developing in a 30 min period. Precipitation of the dye solution still occurred (Fig 48a) and produced a background coloration, nevertheless after 5 hours incubation a faint outline of the 10pg dot could be observed. The dye precipitation was subsequently delayed by prewarming the dye solution to 37°C before use, however dye precipitation still occurred in approximately 40-45 mins.

Figure 48a

Dot-blots of the standard (M13) DNA as detected by the BRL detection system showing precipitation of the dye solutions.



(b) Colorimetric detection of biotin-labelled nucleic acid probes after hybridisation to immobilised nucleic acids

Purified pBR322 DNA was nick translated then dotted on to nitrocellulose and developed using the colorimetric procedure in order to determine the labelled fraction of the biotin-DNA probe. The probe was then used at a concentration of 100ng/ul and hybridised to pre-baked filters dotted with dilution buffer, standard biotin-DNA, and pBR322 DNA at concentrations ranging from 120ug-6ug of DNA. The colorimetric detection system was continued as before for a maximum of 5 hours. After 3 hours dots appeared at 120, 60 and 30ug fractions, however it took a further 2 hours development to produce faint dots in the 18 and 12ug fractions. Dye precipitation was again experienced in this and repetitions of this experiment. Southern blots of DNA were also used in the detection procedure (as described in the Materials and Methods section), however no clear results could be obtained due to dye precipitation and high background colour development.

11. Colorimetric detection of photo-biotin-labelled nucleic acids spotted on to nitrocellulose

(a) Sensitivity

Nitrocellulose filters were spotted with dilutions of biotinylated sheared salmon sperm DNA at concentrations ranging from 200-5pg then baked, hybridised and developed using the colorimetric procedure described in the Materials and Methods section. Within 30 mins the 50pg dot was fully developed, with the 5pg dot

developing in approximately 2 hours. No background coloration was observed and no dye precipitation occurred.

(b) Colorimetric detection of nucleic acid probes after hybridisation to immobilised nucleic acids

The specificity of photobiotin was examined by studying the reactions of photobiotin labelled viral nucleic acid probes. The experimental design was as follows:-

(i) Detection of HSV DNA probe binding

Nitrocellulose filters were spotted with: (1) biotinylated M13 DNA (2pg, 1pg), (2) purified standard strain 17syn^{+} (HSV 1) DNA, (3) dilution buffer, (4) uninfected vero cells DNA, (5) clinical samples of HSV 1 DNA, (6) standard strain HG52 (HSV 2).

Figure 48b shows that the HSV 1 photobiotin labelled probe reacted both with HSV 1 and HSV 2 samples but did not react with dilution buffer or uninfected cellular DNA. This result is not surprising considering the homology between the HSV 1 and 2 genomes. The limit of detection for HSV DNA was approximately 10pg. After drying the colour of the dot blots faded, however the results could be recorded accurately by photography under a white light source (Fig 48b).

(ii) Detection of HPV DNA probe binding

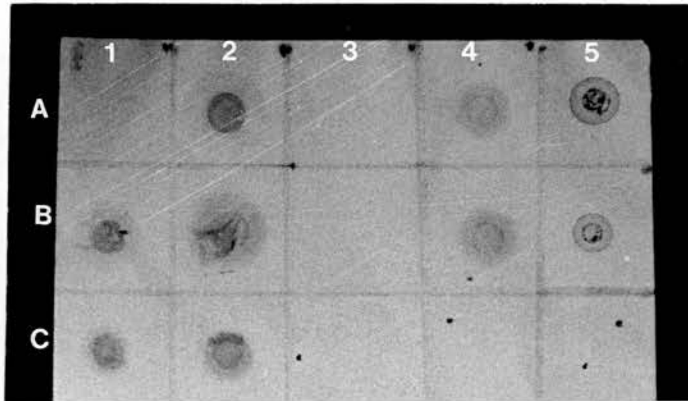
Photobiotin labelled HPV 2 DNA probes were hybridised to: (1) HPV DNA (HPV 1, 2, 6) extracted from clinical wart specimens that had previously been typed by agarose gel electrophoresis and ^{32}P-

Figure 48b

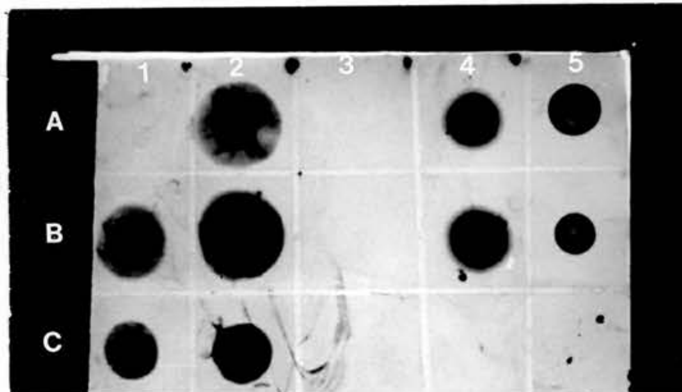
Photobiotin dot blot of HSV DNA, hybridised with standard strain 17syn+ and photographed under incident (a) and transilluminated (b) white light.

Dilution buffer	- A1
17syn+	- A2
Vero cell DNA	- A3, B3
HSV 1 DNA (2028)	- A4, B4
Standard M13 DNA	- A5, B5
HSV 1 DNA (3280, 1728)	- B1, C1
HG52, HSV 2 DNA (11011)	- B2, C2
no samples	- C4, C5, **C3**

a



b



labelled probes, (2) pBR322 DNA, (3) dilution buffer, (4) biotinylated M13 DNA.

Figure 49 shows that the HPV 2 photobiotin labelled probe reacted both with its vector, pBR322 DNA, and the DNA previously typed as HPV 2 DNA extracted from wart specimens. the HPV 2 probe was able to detect 5pg of HPV DNA in clinical specimens.

(iii) Detection of CMV probe binding

Figure 50 demonstrates that the purified HindIII 1 fragment of CMV reacted specifically with CMV DNA isolated from urine specimens that had previously been identified by cell culture. In each case for the clinical CMV specimens the DNA of approximately 10^5 cells (50ng) were spotted on to the nitrocellulose. Samples VS and JB gave very weak results with the detection procedure. No reaction was observed with HSV or uninfected HEF cells.

(iv) Detection of HBV probe binding

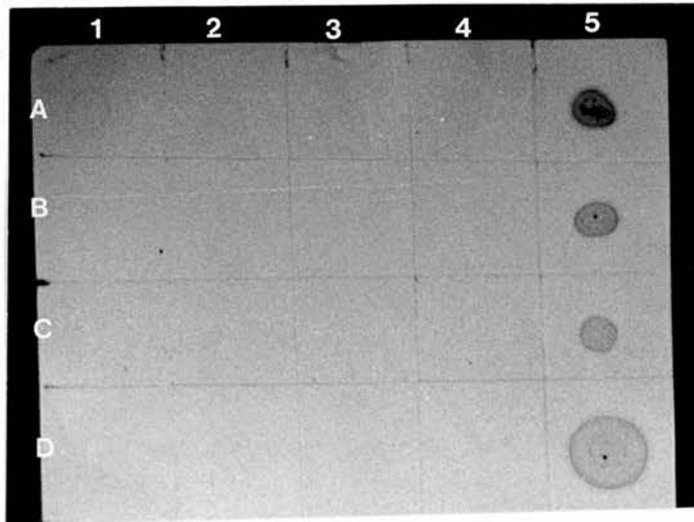
HBV-DNA was hybridised to two positive HBV samples (R1529, R1524) and one negative sample (R1123) provided by the Hepatitis Reference Laboratory. After preparation and purification of the DNA (as described in the Materials and Methods) an average of 0.01ug of DNA was isolated from each sample. Dilutions of 10pg, 5pg, 2pg amounts were spotted on to nitrocellulose filters, as was dilution buffer. After hybridisation with HBV clone and development in the dye solutions for 2hrs the 10pg dot appeared in the positive sample R1529. A faint result was obtained for R1524 after a further hours development in the dye solution. No reaction was apparent for the negative sample R1123 or the dye solution (results not shown).

Figure 49

Photobiotin dot blot of HPV DNA, hybridised with HPV 2 clone and photographed under incident (a) and transilluminated (b) white light.

A1, 2, 3	- HPV 2 DNA	C3	- sample R (HPV 1)
A4	- pBR322 DNA	C4	- sample P (HPV 1)
A, B, CD5	- standard M13 DNA	D1	- sample M (HPV 2)
B1, 2, 3	- wart sample HE (HPV 6)	D2	- sample B (HPV 2)
B4	- wart sample ZA (HPV6)	D3, 4	- no sample
C1, 2	- dilution buffer		

a



b

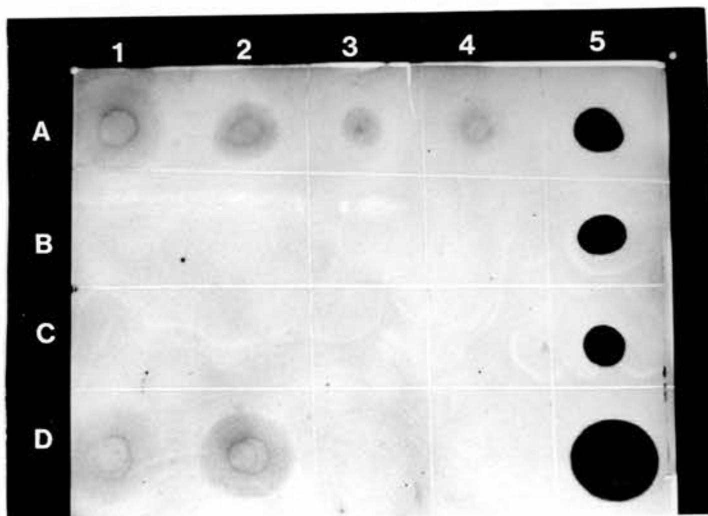


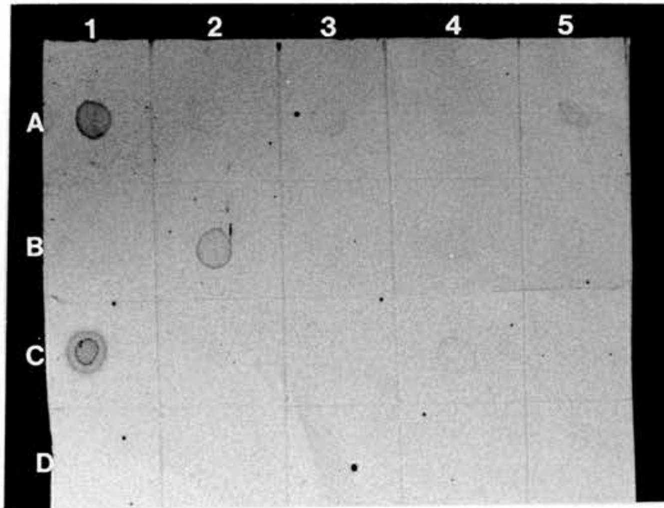
Figure 50

Photobiotin dot bot of CMV DNA, hybridised with HindIII-l fragment and photographed under incident (a) and transilluminated (b) white light.

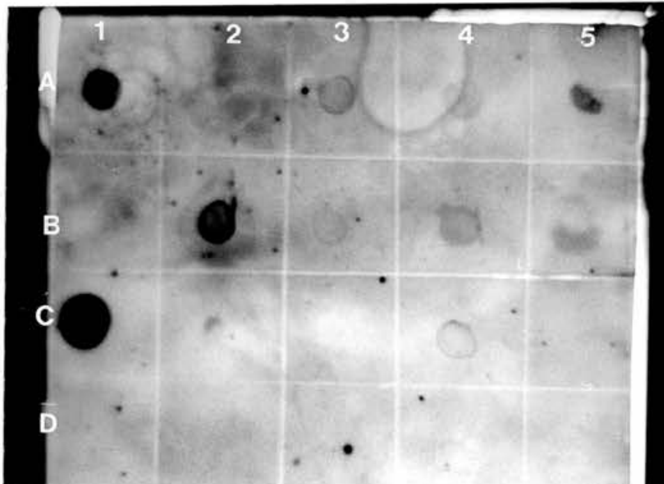
A1 - sample MB
A2 - sample VS
A3 - sample KMc
B1 - sample JB
B2 - sample WS
B3 - sample PW

C1 - standard AD169 DNA
C2 - HSV 1 DNA
C3 - HSV 2 DNA
D1 - dilution buffer
D2 - cell pellet

a



b



DISCUSSION

1. Microcarrier Cell Culture

The production of HSV DNA by microcarrier cell culture proved to be a costly and time consuming when compared with traditional methods of cell culture (Table 4). Microcarrier culture is advantageous in that the cells are cultured as monolayers on small particles suspended in growth medium, therefore large areas can be cultured in a small volume. However, to obtain optimum yields and enhance growth the cultures require constant stirring as well as a rich medium supplemented with 10% calf serum. During the growth of the cells in any culture system the concentrations of various essential components (glutamine, glucose, etc.) are consumed at different rates and require to be replaced by a complete medium change which also helps to maintain the optimum pH. However, in microcarrier culture vessels this process requires a large volume of medium (Table 4). The attachment of cells to microcarriers is a fairly slow process dependent on the number of effective collisions between the cells and the beads. The multiplication rate is, however, only slightly greater than that in stationery culture as shown in Table 4. Unlike stationery cultures in Roux bottles, the microcarrier cell culture required 5-6 days before a monolayer was obtained (Table 4).

After inoculation with HSV (0.1 pfu/cell) a CPE was observed in six days. The total time required for the development of a CPE is obviously important in the production of non-defective HSV DNA, since the yield of HSV DNA/cell for microcarrier culture is poor compared with standard culture in Roux bottles (Table 4). The

infection of the cells on the microcarriers, using a constant virus inoculum and volume, must be dependent on the number of collisons with the cells. If this is the case, all the cells cannot be infected at the same time, since the microcarrier beads have a large surface area.

During the 6 days necessary for the production of a CPE several cells had become detached from the beads. This may explain the relatively poor average yield of DNA/cell obtained by microcarrier cell culture compared to the more conventional Roux bottle production (Table 4). In an attempt to decrease the time taken to produce a CPE in microcarrier culture, the MOI was increased to 1 pfu/cell. Although a CPE was observed in 4 days this method produced large quantities of defective DNA which was unsuitable for use in RE analysis. The production of large amounts of defective DNA in microcarrier culture is comparable to that seen in static culture methods when a high MOI is used.

2. Restriction Endonuclease Analysis

Restriction endonucleases have provided an invaluable tool in the study of the molecular epidemiology of the herpes viruses, allowing differentiation of subtypes (Allen _et al_., 1983a; Pirtle _et al_., 1984) and epidemiological tracing of virus (Buchman _et al_., 1978, 1979; Brown, 1980; Huang _et al_., 1980; Sakaoka _et al_., 1984).

In herpes viruses variations in RE patterns are considered to occur in two forms:

(i) major variations corresponding to the addition or deletion of restriction endonuclease sites

or

(ii) minor variations attributed mainly to mobility differences.

For epidemiological studies much controversy exists about the reliability of using mobility differences for determining epidemiological relatedness of isolates (Smith _et al_., 1981; Roizman and Tognon, 1982; Barton _et al_., 1985). Various laboratories have reported alterations in the RE cleavage of herpes virus DNAs after passage in cell culture (Huang _et al_., 1980; Hirai _et al_., 1981; Zweerink _et al_., 1981; Roizman and Tognon, 1983). These changes appeared as variations in the electrophoretic mobilities of restriction fragments occurring mainly at the joint and terminal regions of the genomes. Similar studies in PRV have also indicated that mobility variations (ranging from 100-400Kb) can be detected with non-plaque purified strains after _in vitro_ (Allen _et al_., 1983b) or _in vivo_ passage (Mengeling _et al_., 1983; Wathen and Pirtle, 1984). However, genomic changes resulting from loss or gain of sites during serial passage have not been reported (Roizman and Tognon, 1982; Allen _et al_., 1983b).

The results of the study in Edinburgh are in agreement with the findings of the aforementioned authors. Examination of the reproducibility of the electrophoretic patterns of standard strain HSV DNA, both from a single preparation and after serial passage in

Vero cells, revealed no variations in the major RE profiles. However, mobility variations of RE fragments were observed mainly from the junction of the genome (e.g. Bam g (HSV 2) K (HSV 1)) (see Results Section 2).

I Genital HSV isolations from a single clinical centre

(a) Source of isolates

Despite numerous epidemiological studies of HSV (Buchman et al., 1978 and 1979; Halperin et al., 1980; Hammerberg et al., 1983) most groups have either examined a small number of isolates (Table 2) or have concentrated on HSV 1 strains (Chaney et al., 1983b; Sakaoka et al., 1984 and 1985). At present only two large scale studies of HSV 2 strains have been attempted. The first by Maitland et al. (1982) considered 65 genital specimens isolated sequentially from 30 patients attending a single clinical centre (Royal Infirmary, Edinburgh (RIE)). However, the second by Chaney et al. (1983a) involved only 38 patients from two geographically distinct areas in Canada.

In comparison to other authors, this study examines the DNA sequence complexity of 224 strains of HSV (180 HSV 2, 44 HSV 1) isolated from 224 patients who attended the Department of Genito-urinary Medicine (RIE) between 1.1.82 and 31.7.83. The proportion of genital HSV 1 isolates (20%) during this time period is in good agreement with previous published figures for the Edinburgh area (Smith et al., 1973). As HSV 1 was isolated more often from females (Smith et al., 1973) than males during the study period it may be of

epidemiologicaly significance, since HSV infection is presumably derived from saliva by oro-genital contact.

Although this project was not set up as an epidemiological study, it was observed that greatest number of isolations appeared to occur in June and July 1982 for HSV 1 and HSV 2 isolates respectively (Figure 15). This is somewhat surprising since Edinburgh experiences a large influx of visitors in August during the International Festival, therefore August and September would have been expected to be the prime months of isolation. This was certainly the case for Neisseria gonorrhoea specimens isolated from patients attending the Department of Genito-urinary Medicine (RIE) during 1982 (Reid, 1985).

The site of isolation detailed by the request forms (Tables 5, 6a) showed that the prepuce was the most common site of isolation (both for HSV 1 and HSV 2) in male patients. For the female patients the isolates were most frequently obtained from the vulva and the cervix for HSV 1 and HSV 2 isolations respectively. However, the analysis is complicated by the fact that isolations were attempted from the cervix and from clinical sites of infection (Table 6b). In some cases the cervix alone was tested in the absence of signs of genital lesions (Table 6b).

(b) Restriction Enzyme Analysis

Digestion of all viral DNA samples with five restriction endonucleases (EcoRI, BglII, HindIII, KpnI, BamHI), for which published RE maps are available, allowed the DNA sequence varations

to be mapped to specific locations on the genome. More importantly, use of RE maps eliminated any possibility of misinterpreting heterogeneity of the junction and terminal fragments as major variations.

The results of the analysis confirm those of previous studies (Maitland et al., 1982; Sheppard et al., 1982; Chaney et al., 1983a) in that the HSV 2 DNA showed relatively infrequent variation, with either one or two site deletions/additions being found with each RE (Tables 7, 9a and b). Greater variation was detected with the multicut endonuclease BamHI, but the majority of these variations were found to be in the terminal or sub-terminal fragments.

The complexity of the analysis with multicut endonucleases emphasises the need for published RE maps when attempting epidemiological analysis. Strain differentiation in this study was therefore based solely on the presence or absence of specific restriction sites and not on the variations in the mobility of RE fragments, although mobility variations were recorded as shown in Figures 29 and 30.

(i) HSV isolates

The HSV 2 isolates from 180 patients were ranked according to their digestion patterns with four enzymes (EcoRI, BglII, HindIII, KpnI) then subdivided by BamHI digestion (Table 9a). Within the population studied the pattern pppx predominates (65 isolates) with the subdivision for BamHI w$^-$ being the most common (35 isolates). This is contrary to a previous study of the Edinburgh area (Maitland

et al., 1982) when xppx appeared to be the most common RE pattern, occurring in 13 of the 30 patients examined (Table A, Appendix). In this instance no classification for the BamHI w⁻ subdivision was attempted

The present study of 180 patients should reflect a more accurate analysis of strain variation within the Edinburgh population. Maitland et al. (1982) also considered that the linkage of EcoRI f-j with KpnI d-i structure may be important in the Edinburgh area. Indeed, comparison of the results of this study and those of Maitland et al. (1982) revealed that 56% of the isolates had both f-j and d-i fusions, while the sample of Chaney et al. (1983a) had only 47% of the isolates with this linkage (Table B, Appendix).

Certain configurations are predominant within the Edinburgh area. The frequency of RE variations (Table B, Appendix) found during the study indicates that the most frequent major variation is KpnI d-i fusion, occurring in 81% of the isolates. This is similar to the 83% reported by Maitland et al. (1982) and slightly less than the 89% of Chaney et al. (1983) (Table B, Appendix). In all three studies the BglII restriction endonuclease sites were the most invariable. It should be noted that several major variations were detected during the course of this study that have not previously been reported (Table 9b). These variations have been confirmed by molecular hybridisation experiments and increase the total number of variable sites in HSV 2 DNA to nine, as detected by the five restriction endonucleases stated (Table 7).

(ii) HSV 1 isolates

All 44 HSV 1 isolates were found to have unique RE profiles as determined by the five restriction endonucleases. A total of 21 major variations were detected (Table 8); less than the total found by Chaney *et al.* (1983b) with the same enzymes. This may be a reflection of the sensitivity of the ^{32}P labelled DNA techniques (Lonsdale *et al.*, 1979) compared to that of the sodium iodide purification (Walboomers and ter Shegget, 1976) used in the present study. However, it may also be due to geographical variation since Chaney *et al.* (1983b) studied HSV isolated from Canadian patients. As with the HSV 2 isolates *BglII* restriction patterns were relatively invariable. The most common site variation was found in 95.4% of the isolates occurring at the *KpnI* s-d site (Table 8). This is marginally less than the frequency reported by Chaney *et al.* (1983b) (Table C Appendix). Comparisons of the variations found in HSV 1 isolates from the Edinburgh area with those of Chaney *et al.* (1983b) are shown in Table C (Appendix). As in previous studies different frequencies of variation are found among the isolates from different geographic locations.

The distribution of variable sites in HSV 1 and HSV 2 isolates were compared for isolations from males and females as well as for anatomical sites (genital/extragenital). However, no differences in the RE site distributions were observed either for HSV 1 or HSV 2 isolations.

II Mobility variations

Variable length fragments are frequently used in the identification of epidemiologically related strains and to distinguish epidemiologically unrelated HSV 2 strains (Chaney _et al_, 1983a; Davis _et al_., 1985). During the study of 224 epidemiologically unrelated HSV isolates mobility variations were found mainly in fragments that occur in the terminal or junction regions of the genome (Figs 29, 30, 33, 34). However, other variable fragments were noted in the unique long and short regions of the genome (Figs 29, 30). Such variations have previously been observed both in cloned stocks of the same virus strain (Roizman and Tognon, 1982) and in clinical isolates (Chaney _et al_., 1983a). Chaney _et al_. (1983a) used variable length fragments _BglII_ i and _BamHI_ y (fragments that are not observed to differ in cloned stocks) in an attempt to distinguish 30 HSV 2 isolates. The authors expected that the length of these variable fragments could help to define strain relatedness. However, comparison of isolates by geographical origin and sex of patient provided no such information regarding either variable length fragments or RE site distribution.

Similar observations have also been made with several other herpes viruses (Allen _et al_. 1983a; Mengeling _et al_., 1983; Pirtle _et al_., 1984) and have led the the authors to believe that variable length fragments should not be used as a criteria for differentiation (Roizman and Tognon, 1982 and 1983).

In an attempt to study the extent of variation in the electrophoretic mobility of restriction endonuclease fragments with site and/or time, selected groups of DNA preparations were examined (Tables 10, 11, 12). In all but one patient the major RE profiles of concurrent and sequential isolates were identical. The one exception occurred in patient M3493 (Table 12) where sequential genital HSV 1 isolates (obtained within a 5.9 year interval) were observed to vary in the KpnI m site. In this instance the patient M3493 did not have reactivation of a latent virus but had been reinfected with a different HSV 1 strain.

Mobility variations occurring mainly in the joint or terminal fragments were also noted between sequential isolates. In one case (E1593, Table 12) the decreased electrophoretic mobility of KpnI e fragment could easily have been misinterpreted as a major variation. The decreased electrophoretic mobility of the KpnI e fragment has previously been reported in plaque purified stocks of standard strain MP-I (Roizman and Tognan, 1983).

III HSV in sexual consorts

The examination of a closely monitored population of 27 index patients and their sexual consorts provided information on the natural history of HSV infections between known sexual partners. In contrast to work presented by other authors (Davis *et al*., 1985; Mertz *et al*., 1985; Rooney *et al*., 1986; Table 2) this study considers 54 patients from whom clearly defined clinical histories had been obtained and immunological status established (Tables 13, 17, 19, 20).

In an attempt to trace the transmission of virus between sexual consorts, all 54 isolates (42 HSV 2, 12 HSV 1) were intially examined 'blind' without consideration of clinical or serological data. By using only major variations the REA could easily pair 10 of the 12 HSV 1 isolates (Table 14). Consultation of the clinical and serological data revealed that the two isolates (1728, 2028) with different RE profiles were obtained from a couple who were in a steady sexual relationship. In this instance the male patient, with non-primary serology, suffered oral herpes 28 days before isolation of virus 2028 from the penis. The female partner, from whom HSV had been isolated from a vulval lesion, may have been infected from the oral lesion of the male during oro-genital sex (Table 13). If this was the case, the HSV isolated from the penis of the male patient would be a reactivation of a latent virus and therefore would be a different infection from that observed in the female partner. Isolations of concurrent and concomitant HSV infections have previously been reported (both for Type 1 and 2 HSV) by other authors (Maitland _et al._, 1982 ; Lewis _et al._, 1984).

Epidemiological tracing of the 42 HSV 2 viruses proved to be much more difficult due to the lack of variable RE sites in the HSV 2 genome (Table 15). In order to clarify the relationship of some of the virus isolates, the clinical and serological data were examined but it became apparent that only 13 of the 21 sexual pairs could reliably be used for the purpose of epidemiological tracing (Tables 16, 17, 18, 19, 20).

Eight pairs of sexual consorts admitted to have had a sexual relationship with other unnamed individuals (Tables 16, 17, 18). The remaining 13 pairs were from stable relationships, with no other sexual partners admitted (Tables 19, 20). In four of the eight cases (Tables 16, 17), where there was evidence of sexual relationships with unnamed individuals, the index patients were found to have primary serology. In one instance (Table 17) both the index patient and the regular sexual consort had primary serology. All four pairs were found to have the same major RE profile as that of their respective named sexual consort, despite having had intercourse with unknown individuals. The source patients of the remaining four pairs of consorts involved in sexual relationships with individuals other than the named partner, had non-primary serology (Table 18). Two of the four pairs had identical RE profiles, however the remaining four isolates (1333/13468, 12667/11259) from two pairs of known consorts, had entirely different RE profiles. Presumably in these cases the infection occurred either by transmission from a previous sexual relationship or by reactivation of latent virus strains.

Restriction enzyme analysis of the HSV isolates from the 13 couples who were involved in stable sexual relationships revealed that 12 of the 13 pairs had identical RE profiles (Tables 19, 20). The remaining couple (3561/4989) (Table 19) despite having had no other recent sexual involvement had different RE profiles. Since both individuals had non-primary serology, presumably each was infected by their own latent virus which had been acquired previously.

Other authors have examined HSV transmission in sexual consorts using RE analysis (Table 2) but only one group, Mertz _et al_. (1985), considered the patients' clinical and serological status. This may not be of particular importance in HSV 1 infections where restriction endonucleases can detect a substantial number of major genomic variations, that enable epidemiological distinction to be made between unrelated isolates. However, with HSV 2 infections, specific epidemiology by RE analysis is much more difficult due to the stability of the HSV 2 genome (see Section 2 Results). By use of multicut endonucleases, analysis of the genome is increased but is accompanied by an increase in the number of minor heterogeneities due to amplification or reduction of the tandemly repeated sequences (Roizman and Tognon, 1982; Roizman and Tognon, 1983; Hayward _et al_., 1984). In these instances a published genomic map is essential in order to identify the hypervariable fragments and to eliminate the possible misinterpretation of simple mobility differences as major variations. Further verification by nick translation and hybridisation experiments is frequently required particularly in the identification of specific hypervariable fragments (Chaney _et al_., 1983a).

The results of this study and a similar study of CMV by Handsfield _et al_. (1985) highlight the need for caution in the interpretation of RE profiles. Isolates from known sexual consorts involved in a stable sexual relationship may exhibit minor heterogeneities (Fig 33) to such an extent that epidemiological tracing may prove to be extremely difficult (Fig 34). It is, of course, to be expected that some sexual partners attending sexually

transmitted disease clinics will be infected with a different viral strain, but without detailed clinical and serological data the source of infection cannot fully be ascertained (Tables 13, 16, 17, 18) (Handsfield *et al*., 1985; Rooney *et al*., 1986). However, if serological and clinical data are known to be reliable then one cannot rule out the possibility that minor mutations may occur after transmission to a new host (Table 19). This has previously been demonstrated in animal viruses after *in vivo* passage of non-plaque purified strains (Mengeling *et al*., 1983; Wathen and Pirtle, 1984).

IV Oro-genital HSV

An increase in the rate of oro-genital contact among the homosexual and heterosexual populations is well documented and has been accompanied by a rise in the isolation of genital HSV 1 (Chacko *et al*., 1982; Corey *et al*., 1983; Soendjojo, 1983). To date very few cases of oral HSV 2 infections have been reported (Kaufman and Rawls, 1972 ; Nahmias and Roizman, 1973 ; Wolonitis and Jeansson, 1977 ; Corey *et al*., 1983 giving rise to the suggestion that previous oral infection with HSV 1, or prior genital infection with HSV 2, may protect against an initial HSV 2 infection of the oral cavity (Corey *et al*., 1983; Docherty *et al*., 1984). In support of this theory is the fact that the majority of oral HSV 2 cases documented have been primary in nature (Corey *et al*., 1983).

The results of this Edinburgh study differ significantly from those of other authors in that 5 HSV 1 and 5 HSV 2 oral and genital isolations were obtained from 10 male patients. As no isolations

were obtained from female patients it may indicate differences in sexual behaviour or simply be a reflection of the population sampled.

All HSV 1 specimens were isolated from primary infections of male heterosexual patients. The HSV 2 specimens were isolated from 3 heterosexual and 2 homosexual males having primary or intial serology with one exception (P5612, Table 22). In this instance the patient (P5612) was found to have had antibody to HSV 2 one year previously, although it is not certain whether this was a first infection of the oral region. Despite this, it would appear that prior infection with HSV did not offer protection against the development of oral HSV 2 for this patient (P5612). All 10 patients in this study had clinical signs and symptoms of herpetic infection, as did the patients examined by other authors (Corey et al., 1983; Docherty et al., 1985). However, there is increasing evidence to suggest that asymptomatic transmission of HSV can occur (Mertz et al., 1985; Embil et al., 1986). This is certainly the case in Neisseria gonorrhoea (Weisner et al., 1973; Young and Bain, 1983), and Chlamydia trachomatis (Goldmeir and Darougar, 1977; Schachter, 1978) isolated from the pharynx, although it has been suggested that the pharyngeal mucosa is not as suited to colonisation as the urethra or cervix (McMillan et al., 1981).

One further theory relating to the lack of oral HSV 2 infection is the presence of saliva. It has been reported that saliva contains not only bacteriocidal factors but also a substance which reduces the susceptibility of the cells to infection by HSV 1 (Heineman and Greenburg, 1980; Ross and Reith, 1985). If the

inhibitory factors have the ability to block or limit the infection of the oral mucosa this would explain the detection of HSV in the saliva of patients who had no active lesions (Douglas and Couch, 1970). Similarly, if the process of blocking is specific or more effective for HSV 2 than HSV 1 then this would account for the lack of HSV 2 in the oral cavity.

In light of these theories REA was employed to examine the variations between HSV isolated from oral and genital regions. As with the larger study of genital isolates the REA was able to 'pair' all the HSV 1 oral isolates with that of their respective genital isolate. However, the lack of variation in the HSV 2 enabled only four HSV 2 isolates to be paired with confidence (Table 24). Of greater importance, perhaps, with regard to the apparent lack of oral HSV 2, was the comparison of oro-genital HSV 1 and HSV 2 isolates with those from the genitalia (Tables 25a and b). Only one major difference was observed between the HSV 1 oro-genital isolates since KpnI m (1.8/1.1) was absent in the oro-genital specimens. However, the HSV 2 isolates showed considerable variations between the genital and oro-genital specimens, particularly in the frequency of EcoRI f-j and BamHI w (1/1) sites. It would therefore appear that HSV 2 isolated from concurrent oral and genital lesions differs from purely genital HSV 2 isolates in the variability of the most common RE sites. However, caution is needed in the interpretation of this small number of results. A larger sample would therefore be required before any firm conclusions can be made.

V HSV isolations from patients in London

The results of three large scale studies of Canadian, Japanese and Kenyan isolates have confirmed that the frequency of RE variations in HSV differs with geographical location (Chaney et al., 1983b; Sakaoka et al., 1985 and 1986). Although small in number, only 15, the specimens from London provided an opportunity to examine HSV isolates that were geographically distinct from those of the Edinburgh area. All HSV 2 specimens were isolated from the genital region whereas the HSV 1 specimens were non-genital isolations. Within the group of HSV 1 specimens were isolates from 4 nurses (B13-16) and two patients who may have been implicated in a possible hospital outbreak of HSV.

As in previous studies (Buchman et al., 1980; Halperin et al., 1980) the RE data established that three isolates (B16, 17, 18) had identical RE profiles (Table 26, Figure 36), thereby confirming the suspected nosocomial outbreak in the intensive care unit. The remaining six isolates (3 from the intensive care unit and 3 random isolates) had unique RE profiles and therefore the patients were not infected with the same HSV 1 strain as B16, 17, 18.

The HSV 2 genital isolates displayed no unusual RE variations with only two variable sites found (EcoRI f-j and KpnI d-i) (Table 27). This lack of variability made it impossible for epidemiological distinctions to be made between five of the six HSV 2 isolates. Comparison of the most variable RE sites found in the London and Edinburgh isolates revealed considerable differences (Table 28a and b).

Four major sites (BamHI a (5.8/2.2), d-h, w-j', and KpnI m (2.6/0.4)) were absent in the HSV 1 (non-genital) London isolates, and this may be a reflection of geographical variation (Table 28a), although it is more likely that this simply reflects the small number of isolates sampled. However, if the results are compared to the five HSV 1 oro-genital isolates (Table 25a) it is still apparent that their is variability in the number and frequency of the RE sites. The London non-genital and Edinburgh non-genital (RTR) isolates are compared in the next section. The HSV 2 genital isolates from both London and Edinburgh again displayed frequency variations in the most common RE sites. However, since the number of isolates from London was very small, the results of the RE site distributions cannot be used to establish geographical variations.

VI HSV isolations from renal transplant recipients

Herpes simplex virus is commonly isolated from transplant patients immediately after transplant when the patients are receiving immunosuppressive drug therapy. Previous serological studies (Warrell *et al*., 1980; Walker *et al*., 1982) have established that the majority of HSV infections after transplant are caused by reactivations of a latent virus. However, it has been established that reinfection with a different HSV strain may occur either from the transplanted organ (Berglin *et al*., 1982) or by other means (Maitland *et al*., 1982; Kit *et al*., 1983; Lewis *et al*., 1984).

The present study of renal transplant recipients revealed that HSV was isolated from 54% of the patients after transplant. This is in agreement with the incidence of HSV infection reported in previous studies (Warrell _et al._, 1980 (47%); Walker _et al._, 1982 (52%)) but differs slightly from the results of Ho _et al._ (1983) who reported only 40.4% infection. Consideration of the clinical details of the patients from whom HSV was isolated revealed that 56% were treated with cyclosporine/prednisolone (CsA/P) and the remaining 44% received azothioprine/prednisolone (A/P). This differs from other studies of renal transplant recipients receiving the same drug therapy.

In 1983 Ho _et al._ reported that 40% of the RTR's from whom HSV had been isolated, were treated with CaA/P whereas 53% received A/P therapy. More recent studies by Harris _et al._ (1985) and Najarian _et al._ (1985) have indicated that CsA/P therapy was administered to 21% and 85% (respectively) of transplant recipients with HSV infections. These differences may be due to the sample population and drug dosage. However, from the present study it would appear that those patients receiving CsA/P after renal transplantation usually developed HSV within a shorter time period than those who were treated with the conventional azothioprine/ prednisolone therapy (Figure 37).

During the course of the study three important applications of REA were examined. The first involved the possible transmission of HSV from patient LL to patients EMcI and DC (Figure 38) during treatment in an open ward. However, no evidence of transmission of HSV was apparent since all three individuals had unique HSV 1 RE

profiles. The second was that the kidney itself can be a source of viral infection as previously reported both for CMV (Weirtheim et al., 1983; Grundy et al., 1986) and HSV (Berglin et al., 1982). In this instance, the donor was known to have had antibody to HSV although no virus sample was available for examination. In addition, no pre-transplant serology was available for one of the two recipients.

Restriction endonuclease analysis of the isolates from both recipients (VS and DL) revealed that the kidney could not be the source of infection in both cases (Figure 41). Since patient DL had antibody prior to transplant, it is most likely that the infection was due to reactivation of a latent virus.

The final aspect was the examination of sequential HSV 1 isolates from three patients who shed virus over periods of 6 weeks to 6 months (Figure 42). For two of the three patients, although they differed between patients, the major RE profiles were constant for sequential isolates. However, mobility differences were noted particularly in fragments occurring at the junction and termini of the genome. The remaining patient IG demonstrated a major variation in the KpnI m (2.6/1.1) site of one isolate. In this case the patient may have been infected with another strain of HSV 1 and therefore had two separate HSV infections during the period sampled. This has previously been reported for both type 1 and type 2 HSV infections by several authors (Maitland et al., 1982; Kit et al., 1983; Lewis et al., 1984) and in previous sections (Sections 4 and 5 of the Results).

A direct comparison between HSV 1 isolates from different anatomical sites, but a single geographical location (Table 30) can be made by examining the distribution of variable RE sites found in the HSV DNA from renal transplant recipients and those of genital isolates. Several sites demonstrated very similar frequencies (e.g. BamHI a (5.2/2.2), BamHI d-h, KpnI m (2.8/1.1), however KpnI m (2.6/0.4) appeared to occur more often in (non-genital) oral HSV isolations than in the genital HSV 1 samples. Although the differences in frequency distribution may simply reflect the small number of non-genital (RTR) patients sampled.

Consideration of Tables 28 and 30 shows that the 16 non-genital isolates from Edinburgh and the seven non-genital isolates from London vary considerably in the frequency of variable RE sites. The London isolates lack BamHI a (5.8/2.2), w-j' and KpnI m (2.6/0.4) sites and show similarity in the frequency of only one site, BamHI d-h. Obviously, no conclusions can be drawn from this comparison due to the small number sampled. It should be noted that other authors (Chaney *et al.*, 1983b) have compared very small numbers of isolates both for anatomical and geographical distribution. Although Chaney *et al.* (1983b) examined the anatomical RE variation in 19 ganglion, 12 genital, 28 facial and 25 encephalitis isolates each group contained a mixture of isolates from geographically distinct areas including Canada, Japan, Britain and the USA.

Summary

(1) Restriction endonuclease analysis of HSV DNA is a useful and reliable means of typing virus isolates. However, for the subtyping of virus isolates caution is needed in the interpretation of the results, particularly when multicut endonucleases are used.

(2) In epidemiological studies any major RE variations should be considered since the use of mobility differences to determine epidemiological relatedness can be misleading. To avoid confusion, analysis should be carried out only with enzymes for which published genomic maps are available.

(3) The variability of the HSV 1 genome and the number of genomic maps available for multicut endonucleases allows epidemiological tracing of HSV 1 isolates. However, analysis of HSV 2 isolates is much more difficult due to the stability of the genome and the lack of published genomic maps. In these instances, where very few major variations are established, it is of particular importance to consider the RE results in light of full clinical and serological status.

3. Polyacrylamide Gel Electrophoresis (PAGE)

Polyacrylamide gel electrophoresis combined with a silver stain was used to examine small molecular weight variations in HSV DNA that could not easily be detected by agarose gel electrophoresis. In the past, several authors have used PAGE to study DNA profiles

for example in 1973, Allett _et al._ detected lambdoid DNA fragments by PAGE using a methylene blue stain. Using a similar technique Favre _et al._ (1977) studied HPV DNA isolated from large quantities of plantar warts. More recently Chaney _et al._ (1983a) and Lewis _et al._ (1984) included PAGE in their analysis of HSV isolates; however neither author reported the type of strain used or the results of the PAGE gels.

The technique used in the present study was ultrasensitive and able to detect small amounts of DNA (~ 1ng). The reproducibility of the technique and limit of detection are in agreement with the results of Herring _et al._ (1982) for rotavirus RNA. Good resolution was achieved with the PAGE gels (Figs 44-47) and thus small molecular weight fragments of 2-0.5kbp could be examined in detail after digestion with multicut endonucleases. In some instances the silver stain detected trace amounts of cellular DNA that was not detected by ethidium bromide staining of agarose gels (Figs 44-47). This may demonstrate that the purification procedure of Walboomers and ter Shegget (1976) does not necessarily eliminate all cellular DNA from an HSV preparation. Although trace amounts of contamination are not detected by ethidium bromide stained agarose gels, cellular contamination may affect the clarity of the results of hybridisation experiments.

Examination of high molecular weight fragments (15Kb-6Kb) was not possible using the PAGE techniques if the smaller molecular weight fragments (4-0.3Kb) were to be analysed. However, these high molecular weight fragments were easily identified by agarose gel electrophoresis.

In order to overcome the difficulties in identifying variable regions in the genome when using PAGE, standard strain DNA was examined and variable fragments recorded. This coupled with a molecular weight marker allowed the identification of variations likely to be caused by junction or terminal fragments of the genome. These regions were observed to vary both in the number and mobility of fragments in epidemiologically related isolates (Fig 44). However, a number of mobility differences were noted between sequential HSV 1 isolates (from RTR's Fig 44, tracks GH and IJ) that could neither be attributed to terminal nor junction fragments. This observation illustrates the need for caution in using mobility as a means of determining epidemiological relatedness.

4. Non-radiolabelled Detection Systems

In recent years DNA probes have provided a useful tool for virological diagnosis as they not only allow analysis from crude sample preparations but also offer a high degree of specificity. Standard detection methods using radioisotopes for dot blot, Southern or *in situ* hybridisation have a number of limitations associated with the problems of safety, stability, storage and disposal of radioisotopes. However, the development of a non-radioactive labelling technique with the vitamin biotin has overcome these problems and has proved to be much more stable than previous methods of preparing non-radiolabelled probes (Miller *et al*., 1966; Renz, 1983; Renz and Kurtz, 1984; Tchen *et al*., 1984).

The original objective to compare the sensitivity and specificity of biotin with photobiotin-labelled DNA probes could not be carried out due to the detection problems encountered with the BRL-biotin kit. Precipitation of the dye solution produced background coloration and prevented the detection of small amounts of DNA. As this occurred both with the manufacturer's biotin labelled sample and with DNA labelled in the laboratory the fault was not due to the nick translation procedure. Filtration of all the solutions and omission of dextran sulphate from the hybridisation mixture did not solve the problem of dye precipitation. Subsequent batches of dye provided by the manufacturer produced slightly better results, however dye precipitation continued. The dye NBT is known to be quite insoluble and has been reported to cause difficulties in detection procedures (Leary _et al_., 1983). On further consultation with the manufacturers it was revealed that other laboratories were experiencing similar problems with dye precipitation. The problem was therefore due either to production or storage of the dye solution by the manufacturer. In any event the biotin detection kit has recently been 'improved' to include a new 'bio-blue gene' detection system.

Investigation of the procedure involving photobiotin used the same NBT dyes as required for the BRL-Biotin KIT but revealed no problems with dye precipitation or visualisation on nitrocellulose. The probe preparation was simple and extremely quick in comparison to the BRL system. However, the biggest advantage of the photobiotin system was that no probe purification or visualisation

processes were necessary to check the nick translation procedure. Instead, the labelled DNA was coloured light orange/red dependent on the amount of DNA present and the position of the lights used for activation. The photobiotin-labelled DNA probes were stable under standard hybridisation conditions (65°C) and gave reproducible results. The BRL-biotin labelled DNA probes were only suitable for medium stringency hybridisation (42°C) and washing procedures. This may, in part, account for non-specific background coloration of the nitrocellulose filters.

Probe specificity was apparent for both biotin and photobiotin labelled nucleic acid probes (Figs 48, 49, 50). The sensitivity of photobiotin as determined by dot blot assay was good enabling the detection of nanogram and picogram quantities. This is similar to the results of Forster _et al_. (1985).

In contrast to techniques employing radiolabels photobiotin-labelled probes are particularly suitable for routine application in clinical laboratories, requiring no specialised containment facilities. Biotin/photobiotin labelled probes have the advantage of being stable for a minimum of one year when stored at -20°C, therefore would be ideal for a diagnostic probe especially for viruses such as HBV and HPV that cannot be grown in cell culture. The application of non-radiolabelled probe technology will in the future provide both a safe and reliable method of monitoring infection by means of the detection of viral nucleic acid in clinical material.

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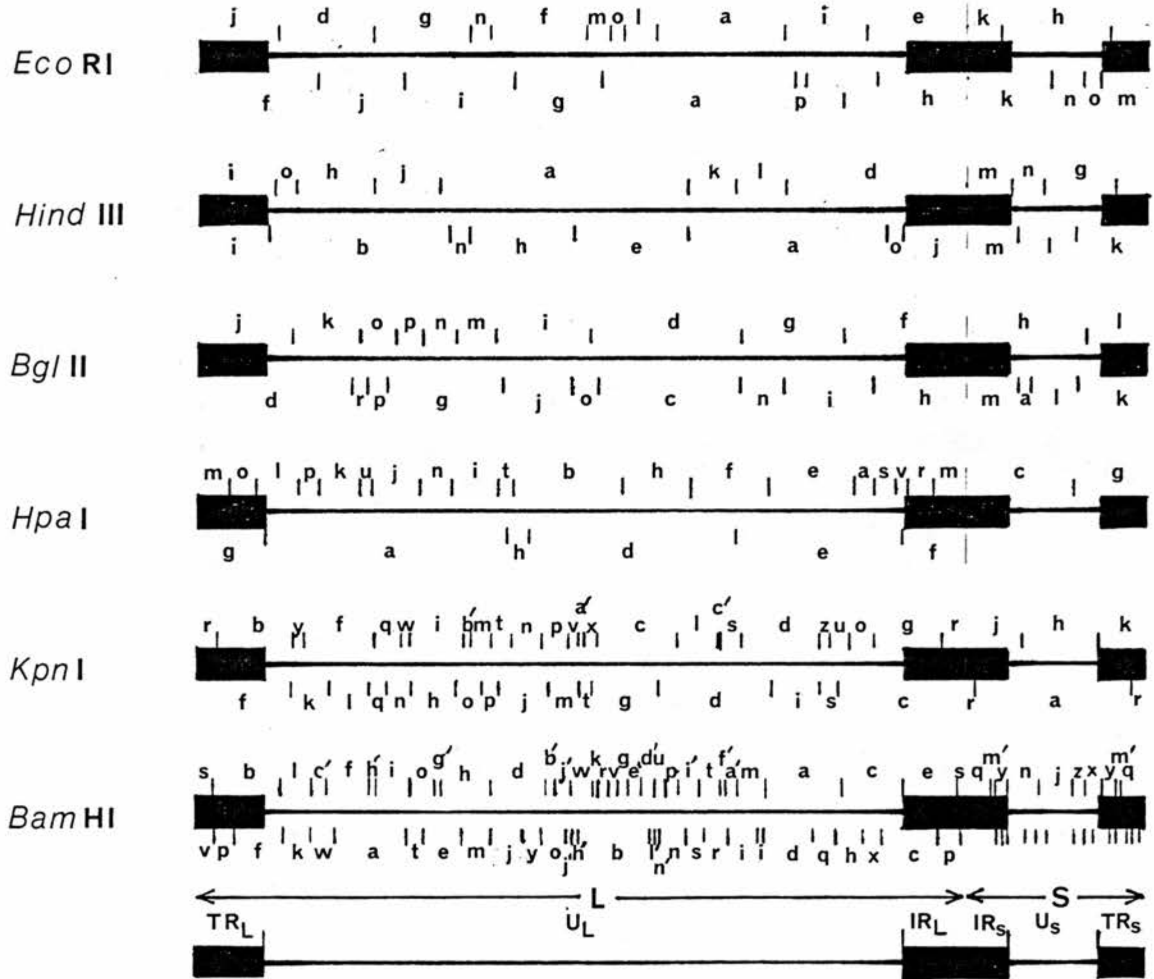
APPENDIX

Figure A

The physical maps of the HSV 1 and HSV 2 genomes.



The HSV 1 maps are shown above whereas those of HSV 2 are shown below and are taken from published data (Cortini and Wilkie, 1978; Wilkie et al., 1978; Davison and Wilkie, 1981 and 1983).

Table A

The frequency of variations in the restriction endonuclease profiles of genital HSV 2 (DNA) found by Maitland *et al.* (1982) and Chaney *et al.* (1983a)*. The Edinburgh results (from Table 9a) are shown in brackets.

Digestion patterns when restricted with: EcoRI BglII HindIII KpnI[a]	(n = 180)	Maitland *et al.* n = 30	Chaney *et al.* n = 38
pppp	(0.11)	0.17	0.08
xppp	(0.07)	0	0.03
xpxx	(0.23)	0.13	0
xppx	(0.31)	0.43	0.47
pxpx	(0)	0	0
pppx	(0.35)	0.23	0.42
ppxx	(0.08)	0.03	0
xxpx	(0.02)	0	0
pxpp	(0)	0.03	0

* The values in the table are x/n where x is the number of isolates having the variable site, and n is the total number of isolates in the group.

a The order of digestion and abbreviations are as in Table 9a.

Table B

The frequency of variable RE sites in genital HSV 2 isolates as found by Maitland *et al*. (1982), Chaney *et al*. (1983a) and the present Edinburgh study.*

Site	Maitland *et al*. n = 30	Chaney *et al*. n = 38	Present Edinburgh Study n = 180
EcoRI f-j	0.56	0.5	0.45
KpnI d-i	0.83	0.89	0.81
BamHI w (1/1)	ND	0.53	0.44

* The values in the tables are x/n where x is the number of isolates having the variable site, and n is the total number of isolates in the group.

ND - not determined.

Table C

The frequency of the most variable RE sites in HSV 1 isolates as determined by Chaney *et al*. (1983b).

		Chaney *et al*. Genital n = 12	Chaney *et al*. Facial n = 28
HindIII	o-h	0.41	0.64
KpnI	s-d	1	1
	b (6.2/1.8)	1	1
	g (5.2/1.8)	1	1
	z-d	0.16	0.14
	m (2.6/0.4)	0.58	0.25
	m (1.8/1.1)	0.16	0.21
BamHI	d-h	0.16	0.21
	w-j'	0.25	0.25
	j'-b'	0.25	0.14
	w-j'-b'	0.08	0
	a (7.2/0.8)	0.66	0.69
	a (5.8/2.2)	0.16	0.07

Table D

Restriction endonuclease data for HSV 1 (DNA) isolated from renal transplant recipients

Patient	EcoRI	BglII	HindIII	KpnI	BamHI
JR	P	P	o-h	s-d m1	a2
DL	P	P	P	s-d g m1	a2
IG	P	P	P	s-d g b m2	a1
VS	K	P	P	s-d b	a1
DC	P	P	P	s-d g b m2	a2 w-j' d-h
EMcI	P	P	P	s-d g b m1	a1 w-j'-b'
TMcG	P	P	m-n	s-d g b m2	a1
LL	P	P	o-h	s-d g b m1	a2
MCL	h-k	P	p	s-d b g m2	a1

MC/....

Table D (continued)

Patient	EcoRI	BglII	HindIII	KpnI	BamHI
MC	P	P	P	s-d b g	a1
HMcK	P	P	P	s-d g b m1	P
AC	P	P	m-n	s-d g b	a1
WS	h-k	P	P	s-d g b	a1
JB	P	P	o-h	s-d g b m1	a1
JC	P	P	o-h	s-d g b m1	w-j'-b'
EB	P	P	P	s-d g b m1	a1

Abbreviations are as in Table 8.

Abbreviations used in clinical and restriction enzyme data of HSV (1 and 2) isolates

Anatomical Sites

BUTT - Buttocks

CX - Cervix

CORSUL - Coronal sulcus

CLIT - Clitoris

FRCT - Fourchette

FREN - Frenum

INT - Introitus

PERIN - Perinum

PREP - Prepuce

RECT - Rectum

VESFLU - Vesicle fluid

NS - Not specified

Restriction Endonuclease Sites

	HSV 2		HSV 1	
EcoRI	EP	- prototype	ERP	- prototype
	EA	- f-j	1	- h-k
			2	- k (2/1.5)
			3	- l-a
BglII	BGP	- prototype	BGP	- prototype
	A	- c (13/3)		
	B	- p-r		
HindIII	HP	- prototype	HDP	- prototype
	A	- e (9/2)	1	- o-h
	B	- m-l	2	- m-n
	C	- a (11/9.5)	3	- k-l

Abbreviations used in clinical and restriction enzyme data

of HSV (1 and 2) isolates (continued)

		HSV 2		HSV 1
KpnI	KP	- prototype	KPP	- s-d
	A	- d-i	1	b (6.2/1.8)
	B	- m (3.1/0.9)	2	- g (5.2/1.8)
			3	- m (2.6/0.4)
			4	- m (1.8/1.1)
			5	- p-v
BamHI	BP	- prototype	BHP	- l (1.7/1.8)
	A	- w (1/1)	1	- a (7.2/0.8)
			2	- a (5.8/2.2)
			3	- d-h
			4	- w-j'
			5	- j'-b'
			6	- w-j'-b'
			7	- o (2.4/0.2)
			8	- m (2.8/0.4)

Clinical and Restriction Enzyme Data of HSV2 (DNA) Isolates

NUMBER	PATNO	SITE	SEX	AGE	DATE	EP	EA	EB	BGP	BGA	BGB	HP	HA	HB	HC	KP	KA	KB	KC	BP	BA	BB	BC	BD
4	M3437	CORSUL	M	31	06/14/83		+		+			+					+			+				
13	P1084	PREP	M	33	06/15/83	+			+			+					+					+		
28	P6279	CORSUL	M	39	06/16/83		+		+			+					+			+				
30	E4557	VULVA	F	25	06/16/83		+		+				+				+					+		
40	A7920	PENIS	M	25	11/25/82		+		+			+					+					+		
121	D3953	FRCT	F	22	12/29/82	+			+			+					+			+				
131	E4577	NS	F	22	06/21/83	+			+				+				+			+				
153	P5113	PREP	M	27	11/30/82	+			+			+				+				+				
154	P5112	URETH	M	31	11/30/82	+			+			+					+			+				
229	M7894	ANUS	M	26	06/23/83	+			+			+					+			+				
259	M8123	ANUS	M	27	12/03/82	+			+			+					+							
316	E3821	VULVA	F	26	12/06/82		+		+			+					+			+				
479	P5202	PENIS	M	25	12/13/82		+		+			+					+					+		
524	P4493	CORSUL	M	29	12/15/82	+			+			+					+					+		
669	D1707	LABIA	F	27	07/11/83		+		+			+					+					+		
678	P5235	PREP	M	23	12/18/82	+			+			+					+			+				
728	E3889	VULVA	F	31	12/23/82		+		+			+					+			+				
732	E1405	LABIA	F	41	12/23/82	+			+			+					+					+		
792	M3634	FRENAL	M	31	12/29/82		+		+			+					+					+		
830	E3203	VULVA	F	21	12/30/82	+			+			+					+			+				
832	E3349	CLIT	F	30	12/31/82		+		+			+					+			+				
858	P6445	PREP	M	27	07/14/83		+		+			+					+			+				
888	P5302	PREP	M	30	01/04/83		+		+			+				+						+		
906	E4666	LABIA	F	18	07/14/83	+			+			+					+			+				
908	P6297	PREP	M	19	07/18/83		+		+			+					+			+				
909	K2685	PREP	M	31	07/18/83	+			+			+					+					+		
933	E3911	LABIA	F	24	01/06/83	+			+			+					+					+		
941	P1684	PREP	M	40	01/06/83	+			+			+					+			+				
952	E4682	FRCT	F	33	07/20/83	+			+			+				+						+		
955	P6476	PREP	M	30	07/20/83		+		+				+				+					+		
999	E905	LABIA	F	31	01/10/83		+		+			+					+			+				
1020	P3268	ANUS	M	28	01/10/83	+			+			+					+			+				
1021	P3585	ANUS	M	32	01/10/83	+			+				+				+			+				
1088	P4920	PREP	M	21	01/13/83	+			+			+				+				+				
1245	E3964	NS	F	24	01/19/83		+		+			+					+					+		
1294	A8364F	NS	F	26	01/20/82	+			+			+					+			+				
1367	P5428	CORSUL	M	18	01/24/83		+		+			+					+			+				
1388	D6980	INTR	F	32	01/25/83		+		+			+				+				+				
1446	P5455	PREP	M	31	01/27/83		+		+			+					+			+				
1654	D6402	LABIA	F	35	02/03/83	+			+			+					+					+		
1662	A8341F	CX	F	22	02/04/83	+			+			+					+					+		
1701	E4046	VULVA	F	19	02/04/83	+			+			+				+				+				
2033	E4105	VULVA	F	19	02/17/83	+			+			+					+			+				
2094	E4116	VULVA	F	26	02/21/83	+			+			+				+						+		
2129	M9269	VESFLU	M	21	02/22/83	+			+			+				+				+				
2135	A8412	NS	M	24	02/23/83	+			+			+					+			+				
2187	E3697	LABIA	F	22	02/24/82	+			+				+				+					+		
2573	MCCARC	NS	F	22	03/10/82		+		+			+					+			+				

Clinical and Restriction Enzyme Data of HSV2 (DNA) Isolates (cont)

NUMBER	PATNO	SITE	SEX	AGE	DATE	EP	EA	EB	BGP	BGA	BGB	HP	HA	HB	HC	KP	KA	KB	KC	BP	BA	BB	BC	BD
2574	HALLIK	NS	F	17	03/10/83	+			+			+				+						+		
2599	E4179	CX	F	21	03/10/83		+		+			+				+				+				
2604	P5687	ANUS	M	31	03/11/83	+			+				+				+					+		
2643	P5178	GLANS	M	30	03/14/83	+			+			+					+					+		
2662	P5718	PENIS	M	21	03/15/83	+			+			+					+			+				
2710	P5721	PREP	M	19	03/15/83	+			+			+					+					+		
2843	P3000	CORSUL	M	34	03/18/83	+			+			+				+				+				
2884	P1274	PENIS	M	20	06/01/81	+			+			+					+					+		
3082	E4250	CX	F	29	03/30/83	+			+			+					+					+		
3097	D4189	ANUS	F	26	07/01/83		+		+			+					+							
3251	P5837	URETH	M	25	04/05/83	+			+			+				+						+		
3361	E4284	LABIA	F	36	04/11/83	+			+				+				+			+				
3528	FIRTHS	VESFLU	M	21	04/15/83		+		+			+					+			+				
3561	M9185	PENIS	M	33	06/25/81	+			+			+				+				+				
3621	K4891	PREP	M	47	04/20/83		+		+			+					+			+				
3700	A8523	NS	M	21	04/22/83		+		+			+					+			+				
3770	E4345	INTR	F	37	04/27/83		+		+			+					+			+				
3828	M6518	URETH	M	31	04/29/83	+			+				+				+					+		
3862	P5988	URETH	M	45	05/03/83	+			+			+					+					+		
4168	A8536	NS	M	23	05/12/83		+		+			+					+			+				
4169	P4384	URETH	M	28	05/12/83		+		+			+					+			+				
4216	E4402	VULVA	F	23	05/12/83	+			+			+					+			+				
4238	E4323	VULVA	F	26	05/13/83		+		+			+				+						+		
4305	E4427	CX	F	22	05/17/83		+		+			+					+					+		
4360	E4432	CX	F	21	05/18/83	+			+			+					+			+				
4826	P5830	PREP	M	26	06/08/83		+		+			+					+			+				
5950	E5424	VULVA	F	22	02/01/84	+			+			+					+					+		
6010	P7732	CORSUL	M	57	02/02/84		+		+			+					+					+		
6060	P4280	PREP	M	22	10/18/82		+		+			+					+			+				
6097	M9838	PREP	M	24	10/19/82	+			+			+					+			+				
6226	M7069	GLANS	M	26	10/25/82	+			+			+				+				+				
6263	P4867	URETH	M	49	10/26/82		+		+			+				+				+				
6443	P4911	FREN	M	31	11/03/82	+			+			+					+					+		
6593	E3234	VULVA	F	22	02/21/84		+		+			+					+			+				
6670	D9692	CX	F	21	11/11/82		+		+			+					+			+				
6675	E3719	CX	F	20	11/11/82	+			+			+					+					+		
6744	P4995	PENIS	M	43	11/12/82		+		+			+					+					+		
6801	E3737	CX	F	30	11/15/82	+			+			+				+						+		
6890	KERRAK	NS	M	25	11/19/82	+			+				+				+			+				
7098	M6180	PENIS	M	35	03/09/84	+			+			+					+					+		
7358	M5833	ANUS	M	34	03/19/84		+		+			+					+			+				
8155	P2900	PREP	M	24	01/06/82		+		+			+					+			+				
8163	M5359	URETH	M	21	01/07/82		+		+			+					+							
8209	P2914	PREP	M	27	01/08/82		+		+			+				+				+				
8370	E2427	CLIT	F	36	01/13/82	+			+			+					+					+		
8386	D578	LABIA	F	47	01/15/82	+			+			+					+			+				
8501	P2962	PREP	M	20	01/19/82		+		+			+				+						+		
8545	E2429	CX	F	19	01/21/82	+			+				+				+					+		

Clinical and Restriction Enzyme Data of HSV2 (DNA) Isolates (cont)

NUMBER	PATNO	SITE	SEX	AGE	DATE	EP	EA	EB	BGP	BGA	BGB	HP	HA	HB	HC	KP	KA	KB	KC	BP	BA	BB	BC	BD
8556	K9224	PREP	M	25	01/21/82		+		+			+					+					+		
8748	M8483	PREP	M	22	01/28/82		+		+			+					+					+		
8751	E2482	VULVA	F	21	01/28/82		+		+			+					+			+				
8762	K9349	PREP	M	30	01/28/82	+			+			+				+						+		
8883	P2346	FREN	M	29	02/03/82	+			+			+					+			+				
8913	P3053	NS	M	31	02/04/83	+			+			+					+			+				
9049	M6890	PENIS	M	23	05/29/79		+		+			+					+			+		+		
9056	D2181	NS	F	33	01/10/82		+		+			+					+					+		
9091	P3097	PENIS	M	24	02/11/82	+			+			+					+					+		
9156	D3612	LABIA	F	25	02/15/82		+		+			+				+				+				
9221	E5523	VULVA	F	24	05/25/84		+		+			+					+			+		+		
9251	P1836	PREP	M	43	02/16/82		+		+			+					+					+		
9252	E1088	CX	F	34	02/16/82		+		+			+					+			+				
9301	D8859	LABIA	F	34	02/18/82	+			+			+					+			+				
9417	H3996	PREP	M	41	02/23/82	+			+				+				+			+				
9616	P2864	PREP	M	46	03/02/82		+		+			+					+					+		
9639	P3238	PENIS	M	29	03/04/82	+			+			+					+					+		
9707	M357	PENIS	M	35	03/05/82	+			+			+					+					+		
9846	D9322	INT	F	28	03/12/82	+			+			+					+			+				
10057	E2692	INT	F	29	03/22/82	+			+				+				+			+				
10079	P3374	URETH	M	34	03/23/82		+		+			+					+			+				
10137	E2289	LABIA	F	33	03/23/82		+		+			+					+			+				
10597	P3498	PENIS	M	35	04/12/82	+			+			+					+					+		
10603	P3501	PREP	M	32	04/13/82	+			+			+					+					+		
10713	P3518	VESFLU	M	38	04/16/82	+			+			+				+				+				
10811	E2826	NS	F	32	04/22/82		+		+			+					+			+				
11008	D6523	LABIA	F	26	04/30/82	+			+			+					+					+		
11011	D9369	ANUS	F	34	04/30/82	+			+			+					+					+		
11071	E2697	CX	F	19	05/05/82	+			+			+				+				+		+		
11073	P1370	PREP	M	23	01/27/82	+			+			+					+					+		
11249	M8943	PENIS	M	22	08/03/84		+		+			+					+			+		+		
11253	P3667	CORSUL	M	24	05/12/82		+		+				+				+			+				
11255	P3670	CORSUL	M	21	05/12/82	+			+			+					+			+				
11256	P4132	NS	M	25	06/15/83		+		+			+					+			+				
11268	P8863	PENIS	M	27	08/06/84	+			+			+					+			+				
11313	E2832	INTR	F	21	05/14/82	+			+			+					+					+		
11331	E2914	VULVA	F	19	05/17/82		+		+				+				+				+			
11356	P3699	PREP	M	27	05/18/82	+			+				+				+			+				
11517	E2940	CX	F	14	05/24/82		+		+			+					+			+				
11628	P3356	CORSUL	M	72	05/27/82	+			+			+					+					+		
11640	E2653	NS	F	33	05/28/82	+			+			+					+					+		
11709	E2971	LABIA	F	32	05/29/82		+		+			+					+			+				
11887	M2267	PREP	M	37	06/08/82	+			+			+					+					+		
11954	P3860	PREP	M	41	06/10/82	+			+			+					+					+		
11984	E3020	CX	F	19	06/11/82		+		+			+					+					+		
12472	P3977	PREP	M	31	07/01/82	+			+			+					+					+		
12473	E1547	NS	F	22	07/11/82	+				+		+					+			+				
12492	E3118	VULVA	F	20	07/02/82	+			+				+				+			+				

Clinical and Restriction Enzyme Data of HSV2 (DNA) Isolates (cont)

NUMBER	PATNO	SITE	SEX	AGE	DATE	EP	EA	EB	BGP	BGA	BGB	HP	HA	HB	HC	KP	KA	KB	KC	BP	BA	BB	BC	BD
12494	E3114	FRCT	F	19	07/02/82	+			+			+					+					+		
12574	P3995	GLANS	M	18	07/05/82		+		+			+					+					+		
12641	E3137	INT	F	21	07/07/82	+					+	+							+	+				
12661	P4030	PREP	M	26	07/08/82		+		+			+				+				+				
12734	P4052	PREP	M	27	07/12/82	+			+			+				+				+				
12746	P4058	GLANS	M	25	07/12/82		+			+		+					+			+				
12753	E1414	PERINM	F	21	07/13/82		+		+			+					+			+				
12756	P4063	PREP	M	30	07/13/82	+			+			+				+						+		
12790	E3166	ANUS	F	21	07/14/82		+		+			+					+			+				
12813	E3171	VULVA	F	23	07/15/82		+		+				+				+			+				
12832	A8041	NS	M	43	07/15/82		+		+			+					+					+		
12928	P4124	PENIS	M	52	07/21/82		+		+					+			+					+		
12929	M7847	PENIS	M	21	07/21/82	+			+			+					+					+		
12993	P4147	PREP	M	23	04/23/82	+			+			+					+					+		
12995	P4144	CORSUL	M	21	07/23/82		+		+				+				+					+		
13078	E1574	FRCT	F	19	07/27/82		+		+			+					+			+				
13111	A7737F	NS	F	21	07/28/82	+			+			+					+			+				
13140	P4177	GLANS	M	25	07/29/82	+			+			+				+				+				
13141	E3215	LABIA	F	19	07/29/82		+		+			+					+			+				
13190	D9353	CX	F	23	07/30/82		+		+			+				+				+				
13226	P4204	PREP	M	33	08/03/82	+			+			+					+					+		
13307	M6693	PREP	M	30	08/05/82		+		+			+					+					+		
13311	P4218	PREP	M	35	08/04/82		+		+			+				+						+		
13364	P2231	ANUS	M	24	08/06/82	+			+			+					+			+				
13365	D5419	CX	F	27	08/09/82	+			+			+					+					+		
13367	H8232	PENIS	M	32	02/14/80		+		+			+				+				+				
13369	E3262	CX	F	31	08/09/82	+			+			+					+			+				
13398	P4259	CORSUL	M	22	08/10/82		+			+		+					+					+		
13398	P4259	CORSUL	M	22	08/10/82	+			+			+					+							
13403	E3279	CX	F	23	08/10/82	+			+				+				+			+				
13468	E3284	LABIA	F	24	08/12/82	+			+			+					+			+				
13470	D3031	CX	F	30	08/16/82	+			+			+					+			+				
13824	E2281	LABIA	F	23	08/31/82		+			+		+					+			+				
13826	P2825	BUTT	M	34	08/31/82	+			+			+				+						+		
13851	D4314	CX	F	23	09/01/82		+		+			+				+				+				
13871	P4446	PREP	M	30	09/02/82	+			+			+					+			+				
13936	P95	PENIS	M	35	09/03/82		+		+			+				+						+		
13941	P4339	THIGH	M	34	09/04/82	+			+			+				+				+				
13993	P4484	PENIS	M	29	09/07/82	+			+			+					+					+		
13996	E3406	CLITOR	F	19	09/07/82		+		+			+					+			+				
14027	P3699	NS	M	38	09/07/82	+			+				+				+					+		
14222	K9835	PREP	M	34	09/15/82		+		+			+					+			+				
14302	D473	LABIA	F	42	09/22/82		+		+				+				+			+				
14357	P4608	PENIS	M	24	09/23/82		+		+			+					+					+		
14557	P4604	NS	M	22	10/01/82		+		+			+					+			+				
14633	E1748	INTR	F	20	10/05/82		+		+			+					+			+				
14927	E3577	CX	F	26	10/14/82		+		+			+					+					+		
14992	P3740	PREP	M	33	10/15/82		+		+			+					+					+		
15900	P9948	PENIS	M	22	01/16/85	+			+			+				+						+		
16092	P9968	ANUS	M	26	01/18/85	+			+			+					+			+				
19296	S592	PREP	M	20	05/02/85	+			+			+				+				+				
1665						+			+			+					+	+				+		
7561							+		+			+			+		+					+		
15762						+					+									+				

Clinical and Restriction Enzyme Data of HSV1 (DNA) Isolates

REC	PATNO	SITE	SEX	AGE	DATE	EFP	ER1	ER2	ER3	ER4	EGP	EG1	EG2	HDP	HD1	HD2	HD3	HD4	HD5	KPP	KP1	KP2	KP3	KP4	KP5	BHP	BH1	BH2	BH3	BH4	BH5	BH6	BH7	BH8
29	E4553	VULVA	F	21	06/16/83	+					+			+						+	+	+		+			+		+	+				
88	E4566	CX	F	23	06/17/83	+					+			+						+	+							+						
231	P6338	PENIS	M	28	06/24/83	+					+			+						+	+	+		+				+			+			
318	E3824	VULVA	F	18	12/07/82	+					+			+						+	+	+						+	+					
374	E3832	PERN	F	23	12/09/82	+					+					+				+	+	+					+			+			+	
609	E3870	VULVA	F	29	12/17/82	+					+			+						+	+	+	+				+		+					
873	P6454	ANUS	M	26	07/15/83	+					+			+						+	+	+					+			+				
961	D9830	NS	F	28	05/03/81					+	+			+						+							+							
1085	E3937	LABIA	F	23	01/12/83	+					+				+					+	+	+							+					
1383	E3982	CX	F	21	01/24/83	+					+				+							+						+		+				
1403	M1693	PENIS	M	30	03/22/77	+					+				+					+	+	+		+				+						
1420	E3936	LABIA	F	32	01/26/83	+					+				+					+	+	+	+				+			+				
1728	E4057	VULVA	F	22	01/24/83	+					+			+						+	+	+	+				+			+				
1827	A8400F	NS	F	20	02/10/83		+				+				+					+	+	+	+				+							
1940	E4088	VULVA	F	22	02/15/83	+					+			+						+	+	+						+				+		
2037	A8410F	NS	F	21	02/17/83	+					+			+						+	+		+					+						
2776	E4199	VULVA	F	18	03/17/83	+					+					+				+	+	+					+						+	
3079	E3227	VULVA	F	23	03/31/83			+			+			+						+	+		+					+		+				
3349	A8837	PENIS	M	16	10/14/83	+					+			+						+	+	+					+		+					
3803	E4347	CX	F	23	04/28/83	+					+			+						+	+	+			+			+	+					
4022	P1752	RECT	M	30	07/13/81	+					+				+					+	+	+	+				+							
4083	P6032	PENIS	M	32	05/04/83	+					+			+						+	+		+						+	+				
4287	E4417	VULVA	F	22	05/16/83	+					+			+						+	+	+		+							+			
4547	E4479	INT	F	20	05/27/83	+					+			+						+		+					+							
4552	E4476	VULVA	F	19	05/27/83	+					+			+						+	+	+					+					+		
4639	M3493	PENIS	M	21	05/25/78	+					+				+					+	+			+			+							
5028	E5309	LABIA	F	23	03/11/82						+			+						+	+	+					+				+			
6075	E1492	CX	F	21	10/19/82	+					+				+					+	+	+		+			+				+			
6446	P7829	PENIS	M	20	02/16/84	+					+			+						+	+	+			+			+						
6509	E3695	VULVA	F	19	11/05/82	+					+				+					+		+						+			+			
6517	E3682	VULVA	F	19	11/05/82	+					+				+					+	+						+		+					
6608	E3706	CX	F	18	11/08/82			+			+				+					+	+	+												
7899	E5303	VULVA	F	30	06/04/84	+					+			+						+	+	+		+			+				+			
8039	P2872	PENIS	M	18	12/30/81	+					+			+						+	+	+					+							
8513	D6440	PERN	F	24	01/20/82			+			+				+					+	+	+		+			+							
8935	E1593	NS	F	19	04/02/82	+					+				+						+	+						+		+				
9058	E2531	LABIA	F	37	02/10/82	+					+			+						+	+	+	+										+	
9302	E2569	VULVA	F	21	02/18/82	+					+			+						+	+	+		+			+		+					
9495	K9816	GLANS	M	16	06/01/76	+					+				+					+	+	+					+							
9606	E2622	LABIA	F	19	03/02/82			+			+			+						+	+	+		+	+			+						+
10525	E2779	VULVA	F	19	12/07/82		+				+			+						+	+	+					+			+				
10874	D5112	VULVA	F	23	04/23/82	+					+				+	+				+	+	+					+			+				
11094	A7948	NS	M	19	05/05/82	+					+			+						+	+	+		+			+			+				
12047	E708	PERN	F	19	06/18/82	+					+				+					+	+	+		+			+				+			
12094	D1800	CX	F	26	06/15/82				+		+			+						+	+	+	+											
12095	P2935	URINE	M	21	06/14/82	+					+			+						+	+	+	+			+								
12201	E3065	LABIA	F	29	06/21/82			+			+			+						+	+	+		+					+					+
13059	E225	ANUS	F	20	07/26/82	+					+			+						+	+	+												
13257	E3251	LABIA	F	26	08/05/82	+					+					+						+					+						+	
13578	P4322	ANUS	M	24	08/19/82	+					+			+						+	+	+			+		+							+
13663	P379	ANUS	M	35	08/25/82		+				+					+				+	+		+		+						+			
13748	D8246	NS	F	23	09/02/82	+					+			+						+	+	+	+				+							
14023	P4488	PREP	M	18	09/07/82			+			+					+				+	+	+			+			+		+				
14277	E1491	VULVA	F	22	09/21/82	+					+			+						+	+	+			+		+							+
14615	K6364	PREP	M	27	10/04/82			+			+			+						+	+	+						+						
14739	E3422	CLIT	F	18	10/08/82	+					+				+		+			+	+	+	+				+							