HERPES SIMPLEX VIRUS-INDUCED GLYCOPROTEINS: MAPPING AND CHARACTERISATION OF g92K AND gE

by

Ralph Graham Hope

A Thesis Presented for the Degree of

Doctor of Philosophy

in

The Faculty of Science

at the University of Glasgow

Institute of Virology
Church Street
GLASGOW
G11 5JR

March 1986

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to thank the following people for their assistance in bringing this thesis to completion:

Professor J.H. Subak-Sharpe for provision of the facilities at the Institute of Virology and for his interest throughout the course of this work.

My supervisor, Dr. Howard S. Marsden, for his excellent and valuable criticism of the manuscript and for his guidance and friendship throughout the time spent working in his laboratory.

Drs. A. Cross, J.W. Palfreyman and A. Minson for their gifts of monoclonal antibodies and Dr. J.W. Palfreyman for his help in the initial immunological experiments.

I thank my friends in laboratory 300 for their encouragement and sense of humour.

Mrs. Agnes J. Simpson for her advice and excellence in typing this manuscript.

I am grateful to John McLauchlan for his assistance in proof-reading the manuscript.

My wife, Maria, for her patience and understanding, my children, Jonathan and Gavin, who always welcomed me home with a smile, and my father whose encouragement is acknowledged.

SUMMARY

Cells infected with HSV-1 (strain 17 syn+) or HSV-2 (strain HG52) incorporated inorganic sulphate into polypeptides which comigrated on SDS polyacrylamide gels with virus-induced glycoproteins. The major sulphated glycoprotein was glycoprotein E. In addition, inorganic sulphate was incorporated into glycoprotein D and HSV-1 glycoproteins B, C and Y. Incorporation of sulphate label into HSV-2 glycoproteins B/C was occasionally observed. The addition of inorganic sulphate occurs late during glycoprotein maturation.

Analysis of intracellular sulphated polypeptides using intertypic recombinants mapped glycoprotein E to between 0.886 and 0.935 map units (mu) of the HSV genome.

Studies from other laboratories had established the types of oligosaccharide linkages on glycoproteins B, C and D. Experiments are reported in this thesis in which the drug tunicamycin was used to investigate the nature of the linkage of oligosaccharides to HSV-1 induced glycoproteins E and Y and the nature of the sulphate linkage to all the recognised HSV-1 glycoproteins. Synthesis of both gE-1 and gY-1 was inhibited by the drug, suggesting they contain N-linked oligosaccharides. Tunicamycin also inhibited the incorporation of inorganic sulphate into all HSV-1 glycoproteins although reduced amounts of sulphate could be detected in an abnormal form of gE-1. These results suggest that most inorganic sulphate appears to be attached to N-linked oligosaccharides but for gE-1 some may be attached to the polypeptide backbone or to O-linked oligosaccharides.

Major sulphated species of apparent MW 32000, 34000 and 35000 were secreted from cells infected with 17 syn+. In addition, sulphated polypeptides which migrated in the vicinity of glycoprotein D were secreted from cells infected with 17 syn+. These species were subsequently shown

by tryptic peptide fingerprinting to be encoded by the gene encoding glycoprotein E. Furthermore, over 95% of the total amount of 32000, 34000 and 35000 polypeptides synthesised was secreted.

The secreted proteins are produced when infected cells are incubated in the presence of serum but are not produced in its absence demonstrating that a serum component is responsible for their generation. Unlike gE, the secreted proteins do not possess affinity for the Fc end of IgG.

Evidence is presented showing that the 92000-dalton glycoprotein (g92K) induced by HSV-2 has properties distinct from those assigned to any other HSV-2 glycoproteins. First, the carbohydrate composition and extent of sulphation differ from those of glycoproteins D and E. Second, two clonally unrelated monoclonal antibodies, AP1 and LP5, shown to specifically immunoprecipitate g92K, do not react with any of the known processed forms of glycoproteins B, C, D and E. Third, by using HSV-1 x HSV-2 intertypic recombinants g92K was shown to map in the short region of the HSV genome (0.846 - 0.924). Fourth, the intertypic recombinant R12-1 which did not induce g92K, induced HSV-2 gE and an altered gD, providing genetic evidence that g92K is encoded, at least in part, by a different region of the genome from that encoding gE.

ABBREVIATIONS

A	adenine
aa	amino acid
AMP	adenosine monophosphate
ara-C	arabinosylcytosine
arg	argenine
BHK	baby hamster kidney
bp	base pair
BSA	bovine serum albumin
C	cytosine
CHO	Chinese hamster ovary
Ci	curie
CMP	cytidine-5'-monophosphate
^{o}C	degrees centigrade
dATP	2'-deoxyadenosine-5'-triphosphate
DNA	deoxyribonucleic acid
dTTP	2'-deoxythymidine-5'-triphosphate
dUMP	2'-deoxyuridine-5'-monophosphate
dUTP	2'-deoxyuridine-5'-triphosphate
dUTPase	2'-deoxyuridine-5'-triphosphate nucleotidohydrolase
EM	electron microscope
endo-D	endo-beta-N-acetylglucosaminidase-D
endo-F	endo-beta-N-acetylglucosaminidase-F
endo-H	endo-beta-N-acetylglucosaminidase-H
ER	endoplasmic reticulum
Fc	fragment crystallizable
FPV	fowl plague virus
G	guanine
galNAc	N-acetylgalactosamine

GDP	guanosine-5'-diphosphate
glcNAc	N-acetylglucosamine
gly	glycine
GuHCl	guanidine hydrochloride
h	hour
HEL	human embryo lung
HeLa	Henrietta Lax
HEp-2	human epithelial
HFL	human foetal lung
his	histidine
HSV	herpes simplex virus
IE	immediate-early
Ig	immunoglobulin
ile	isoleucine
ITMP	integral trans-membrane protein
kb	kilobase pairs
MAB	monoclonal antibody
mann.	mannose
mg	milligram
$MgCl_2$	magnesium chloride
min.	minute
ml	millilitre
moi	multiplicity of infection
mRNA	messenger ribonucleic acid
mu	map units
MW	molecular weight
NAGO	N-acetylgalactosaminyl oligosaccharidase
NaSCN	sodium thiocyanate
NPT	non-permissive temperature
ORF	open reading frame

PAA	phosphonoacetic acid
PAGE	polyacrylamide gel electrophoresis
PEG	polyethylene glycol
pfu	plaque forming units
PI	post-infection
poly A	polyadenylic acid
PT	permissive temperature
RK	rabbit kidney
RNA	ribonucleic acid
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
ser	serine
SFV	semliki forest virus
sial	sialic acid
SRP	signal recognition particle
Staph. aureus	Staphylococcus aureus
SV5	simian virus 5
syn	syncytial
syn^+	non-syncytial
T	thymine
thr	threonine
TK	thymidine kinase
TLCK	N-alpha-p-tosyl-L-lysine chloromethylketone
TM	tunicamycin
TMP	2'-deoxythymidine-5'-monophosphate
TPCK	L-1-tosylamide-2-phenylethylchloromethyl ketone
ts	temperature sensitive
UDP	uridine-5'-diphosphate
V	volts

V_{MW}	molecular weight, in kilodaltons, of herpes virus-induced polypeptides
VSV	vesicular stomatitis virus
v/v	volume/volume (ratio)
w/v	weight/volume (ratio)
w/w	weight/weight (ratio)
uCi	microcurie
2-D	two-dimensional

INTRODUCTION

CHAPTER 1

INTRODUCTION

This introduction is presented in three sections. The first deals with the biology of herpesviruses. The second deals with structure and function of glycoproteins while the third is devoted to herpes simplex virus (HSV) glycoproteins. In that section, their genome locations synthesis, processing and the functions associated with them are discussed in detail.

SECTION A: BIOLOGY OF HERPESVIRUSES

1.1 Classification of herpesviruses

The family HERPES VIRIDAE can be grouped into three subfamilies, ALPHA-, BETA- or GAMMAHERPESVIRINAE (Roizman et al., 1978, 1981; Roizman, 1985; Mathews 1982). A representative list of each is shown in Table 1. ALPHAHERPESVIRINAE and GAMMAHERPESVIRINAE are differentiated according to their host range in vitro and characteristics of latent infection. ALPHAHERPESVIRINAE in vitro have a variable host range, and produce latent infections in ganglia. GAMMAHERPESVIRINAE in vitro replicate in lymphoblastoid cells, being specific for B and T lymphocytes and produce latent infections in lymphoid tissue. Those herpesviruses grouped into the BETAHERPESVIRINAE subfamily have a relatively long reproductive cycle and slow development of cytopathology in cell culture. The ALPHAHERPESVIRINAE have a genome size of 85-110 x 10^6 whereas the BETAHERPESVIRINAE have a genome size of 130-150 x 10^6.

TABLE 1

Herpesviruses

Alphaherpesvirinae	Herpes Simplex Virus 1 (HSV-1)	Gruter (1924)
	Herpes Simplex Virus 2 (HSV-2)	Schneweis (1962)
	Equine Abortion Virus (EHV-1)	Plummer and Waterson (1963)
	Bovine Mammilitis Virus (BMV-2)	Martin et al. (1966)
	Pseudorabies Virus (PrV)	Gustafsohn (1970)
	Channel Catfish Virus (CCV)	Wolf and Darlington (1971)
Betaherpesvirinae	Human Cytomegalovirus (CMV)	Smith (1956)
	Mouse Cytomegalovirus	Smith (1954)
Gammaherpesvirinae	Epstein-Barr Virus (EBV)	Epstein et al. (1965)
	Marek's Disease Virus (MDV)	Churchill and Biggs (1967)

1.2 Structure of the herpesvirion

The herpesvirion contains four morphologically distinct structures:- the core, capsid, tegument and envelope. The core contains a double-stranded DNA genome (Epstein, 1962; Ben-Porat and Kaplan, 1962) which is toroidally arranged around a central proteinaceous matrix (Chai, 1971; Furlong et al., 1972; Nazerian, 1974; Heine and Cottler-Fox, 1975).

The icosahedral capsid which surrounds the core is approximately 100nm in diameter and consists of 162 capsomeres arranged with 5:3:2 axial symmetry (Wildy et al., 1960). The capsomeres of which 150 are hexameric and 12 are pentameric are hollow elongated prisms and intercapsomeric fibrils have been observed (Wildy et al., 1960; Vernon et al., 1974).

A fibrous layer known as the tegument surrounds the capsid (Roizman and Furlong, 1974). The tegument is surrounded by the virion envelope which consists of a trilamellar membrane with spikes about 8-10nm long, projecting from its outer surface (Wildy et al., 1960).

The mature herpesvirion contains approximately 18-33 polypeptides, some of which are phosphorylated or glycosylated, and range in apparent MW from 11000-290000 (Spear and Roizman, 1972; Heine et al., 1974; Gibson and Roizman, 1974; Perdue et al., 1974; Stevely, 1975; Marsden et al., 1976; Dolyniuk et al., 1976). Many of the structural proteins of HSV-1 have been assigned locations within the virus particle on the basis of experiments involving selective removal of polypeptides by detergent treatment (Roizman and Furlong, 1974), differential chemical treatment (Olshevsky and Becker, 1972; Roizman and Furlong, 1974) and virus neutralisation (Powell et al., 1974; Cohen et al., 1978). Early studies suggested that the envelope contains most or all of the virus coded glycoproteins (Spear and Roizman, 1972), cell lipids (Asher et al., 1969) and spermidine (Gibson and Roizman, 1971). More recently, seven major capsid

and electron microscope measurements on partially denatured DNA molecules

polypeptides have been identified in HSV-1, ranging in MW from 12000 to 155000 (Cohen et al., 1980). The 155000 major capsid protein has been shown to participate in disulphide bonding in the virion structure (Zweig et al., 1979). Polypeptides which have not been located in the capsid or envelope are thought to reside in the tegument. $V_{MW}43$ (VP21) appears to be a structural component of the core (Gibson and Roizman, 1972).

1.3 Structure of the HSV genome

The MW of the HSV genome has been determined by sedimentation, electron microscopic and restriction endonuclease analysis (Becker et al., 1968; Kieff et al., 1971; Wilkie, 1973, 1976; Grafstrom et al., 1974; Wadsworth et al., 1975; Clements et al., 1976) to be approximately 98×10^6 daltons. The genome has a deoxyguanosine plus deoxycytosine (G+C) content of 67% (Kieff et al., 1971) compared to a G+C content of 69% for HSV-2 (Goodheart et al., 1968; Kieff et al., 1971; Halliburton, 1972) and in contrast to mammalian cellular DNA, the doublet CpG is not present at a reduced frequency (Subak-Sharpe et al., 1966). Approximately 50% of the HSV-1 and HSV-2 DNA sequences are homologous on the basis of DNA-DNA hybridisation experiments (Kieff et al., 1972; Frenkel et al., 1973; Sugino and Kingsbury, 1976).

The HSV genome consists of two segments, the long (L) and short (S) components, each of which comprises a unique region (U_L and U_S) bounded by inverted repetitions (TR_L and IR_L, TR_S and IR_S) as summarised in fig. 1 (Sheldrick and Berthelot, 1974; Hayward et al., 1975b; Delius and Clements, 1976; Roizman, 1979). Sheldrick and Berthelot (1974) suggested that the L and S segments might invert by intramolecular recombination, thereby generating four distinct genome arrangements. The establishment of restriction endonuclease maps have shown that this does occur and that virion DNA exists as an equimolar population of four DNA

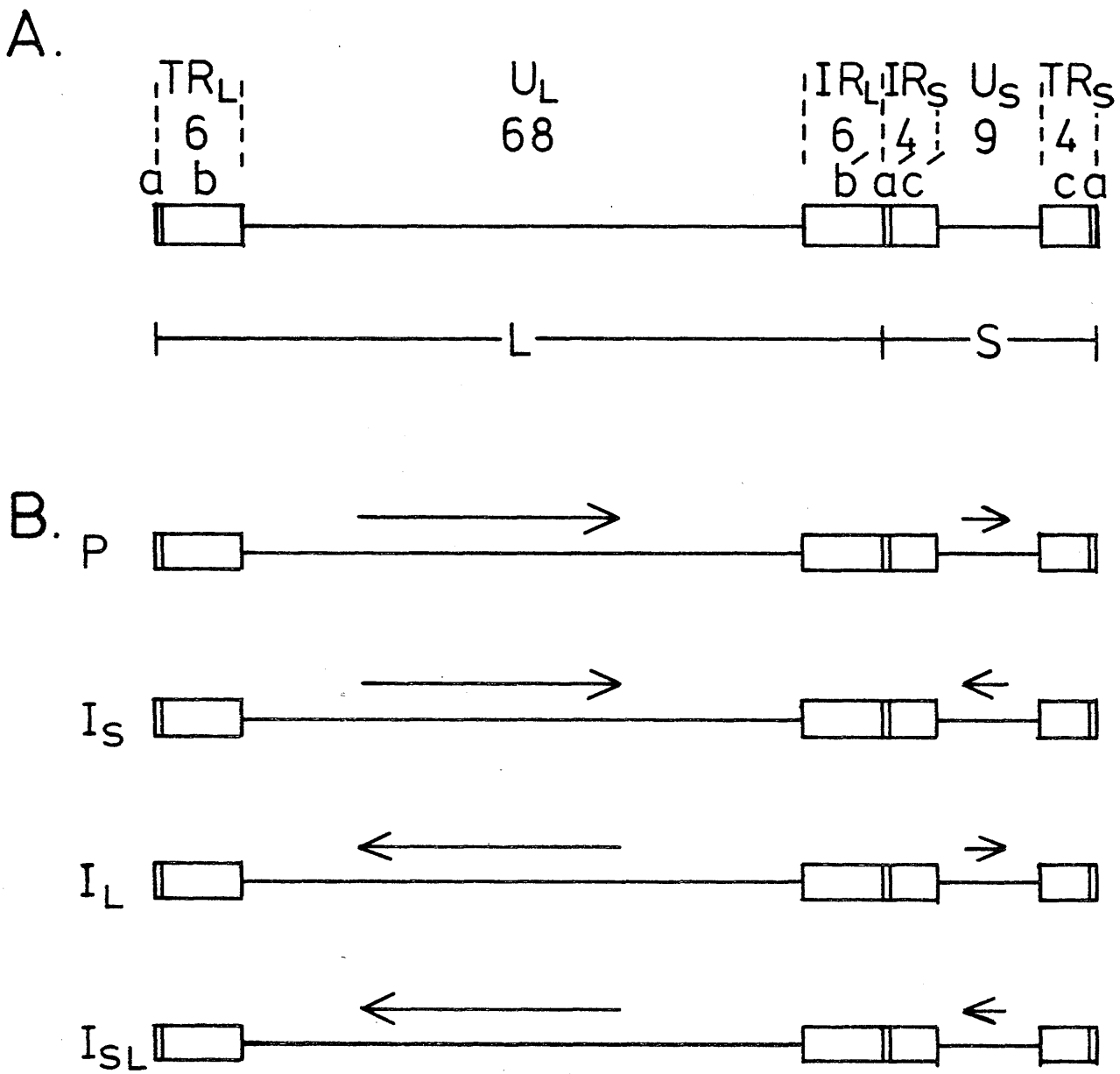

A.
TRL
6
a b
UL
68
IRL
IRS
6
4
b a c
US
9
TRS
4
c a
L
S
B.
P
IS
IL
ISL

FIGURE 1

HSV-1 genome structure:

(A) The genome arrangement of HSV showing the terminal repeat (TR) and internal repeat (IR) sequences of the long (L) and short (S) components. The number above each sequence refers to the size in millions of daltons.

(B) The four genome isomers:

P	Prototype orientation
I_S	Inversion of short region
I_L	Inversion of long region
I_{SL}	Inversion of long and short regions

The arrows show the orientation of the segments.

a,a' The a sequence (a). A sequence of DNA found at the termini of the HSV genome and in an inverted form (b') at the L/S junction.

b,b' A sequence of DNA (b) in TR_L bordering the a sequence and found in inverted form (b') in IR_L.

c,c' A sequence of DNA (c) in TR_S bordering the a sequence and found in inverted form (c')in IR_S.

species which differ only in their relative orientation of the L and S components (fig. 1) (Roizman et al., 1974; Hayward et al., 1975a; Wadsworth et al., 1975; Delius and Clements, 1976; Wilkie and Cortini, 1976; Skare and Summers, 1977).

1.4 The a sequence

The a sequence is present as a direct repeat at each terminus of the DNA molecule and in an inverted form at the L-S joint (Sheldrick and Berthelot, 1974; Grafstrom et al., 1974, 1975). Its size has been estimated at 265bp from restriction endonuclease mapping of the HSV-1 strain KOS (Wagner and Summers, 1978) and 400-1600bp by electron microscopy (Grafstrom et al., 1974, 1975; Wadsworth et al., 1976; Kudler and Hyman, 1979; Davison and Wilkie (1981)) depending on the HSV-1 strain used. The a sequence is bordered by regions which occur at the termini and in inverted form at the joint. These are designated b sequences for those found in the L region and c sequences for those found in the S region (fig. 1) (Sheldrick and Berthelot, 1974; Grafstrom et al., 1974, 1975).

Two types of size heterogeneity in the joint and terminal regions have been described and were originally detected by variable size of restriction endonuclease fragments. One is thought to correspond to the number of a sequences present (Wilkie, 1976; Wilkie et al., 1977; Wagner and Summers, 1978; Locker and Frenkel, 1979), and the other is thought to correspond to smaller multiple tandem repeats of 10-50bp in the a sequence and the adjacent c sequence (Wagner and Summers, 1978).

Nucleotide sequencing data have revealed that the a sequence is flanked by direct repeats and contains internal tandem repeats (Davison and Wilkie, 1981; Mocarski and Roizman, 1981). The a sequence of HSV-1 strain 17 syn$^+$, has the structure (DR1) (Ua) $(DR2)_{18}$ (Ub) (DR1), where DR1 is a 17bp direct repeat, DR2 is a 12bp repeated sequence of which there are

18 copies and Ua and Ub are unique sequences (Davison and Wilkie, 1981). A similar structure, but with two adjacent internal repeats, i.e. (DR1) (Ua) (DR2)m (DR3)n (Ub) (DR1) has been shown for HSV-1 strain F (Mocarski and Roizman, 1981) and HSV-1 strain USA-8 (Davison and Wilkie, 1981).

1.4.1 Functions of the a sequence

It has been demonstrated (Mocarski et al., 1980; Smiley et al., 1981; Mocarski and Roizman, 1982a, b) that the a sequence plays a role in inversion. The a sequence containing fragments from the joint L or S terminus was inserted into the U_L region. This generated additional inversion events of DNA fragments bounded by the inverted a sequence showing that the signal for inversion lies within the a sequence.

Stow et al. (1983) demonstrated that the a sequence contains DNA sequences required for encapsidation of the virus genome. BHK cells were transfected with plasmids containing the HSV-1 origin of replication, then superinfected with HSV-1 as helper. Only replication and concatemer formation of the plasmids occurred. If, however, the plasmids contained, in addition, DNA from the a sequence, replication, concatemer formation and encapsidation occurred allowing further passage of the replicated HSV-1 DNA.

Dalziel and Marsden (1984) presented evidence that the a sequence of HSV-1 strain 17 syn^+ contains DNA sequences that interact specifically with the HSV-1 polypeptides of apparent MW 21000 and 22000 (probably ICP47 and ICP48 respectively). Their observation was based on a DNA competition binding assay using cloned DNAs from different regions of the virus genome to search for HSV-induced proteins which might interact specifically with regions of the HSV genome. The functional significance of this interaction is not yet known.

1.5 Infections by HSV

1.5.1 Lytic infections

Adsorption of the HSV-virion onto a cell is one of the initial events of HSV infection. The viral structure(s) responsible for this event has/have not yet been identified, however as discussed in Section 1.22.2, viral glycoproteins are probably directly involved, in particular, HSV-1 gB and gD (Johnson et al., 1984). Although adsorption can occur at +4°C, penetration requires higher temperatures (Farnham and Newton, 1959; Holmes and Watson, 1963; Huang and Wagner, 1964). Several authors have shown that the viral envelope is required for infectivity (Smith, 1964; Spring and Roizman, 1968; Stein et al., 1970; Rubenstein et al., 1972) and that specific viral receptors are present on the cell surface (Hochberg and Becker, 1968; Blomberg, 1979; Vahlne et al., 1979). Virions of one serotype can block the binding of homologous, but not of heterologous virus (Vahlne et al., 1979, 1980) showing that HSV-1 and HSV-2 recognise different receptors on the cell surface.

Two routes have been proposed by which the virion may penetrate the cell surface. First, EM studies suggested that the virion was taken into the cell, into vacuoles, by pinocytosis (Holmes and Watson, 1963; Hummelar et al., 1969; Dales and Silverberg, 1969). Second, Morgan et al. (1968), also from EM studies, suggested that infectious penetration by HSV may occur by fusion of the virion envelope with the cell surface membrane. Further evidence that the second suggestion is correct has been presented by Sarmiento et al. (1979), Little et al. (1981) and DeLuca et al. (1981) using a HSV-1 mutant which will only enter cells at the non-permissive temperature (NPT) in the presence of the fusion inducing agent, polyethylene glycol. As discussed in more detail in Section 1.22.3, this mutant is blocked in the processing of gB at the NPT, suggesting that gB plays a role in the penetration of HSV-1 into cells.

After penetration of virions into cells in tissue culture, mitosis ceases (Stoker and Newton, 1959; Wildy et al., 1961). Initiation of the infectious cycle is independent of the phase of cellular DNA synthesis (Cohen et al., 1971). Infection with HSV results in inhibition of overall host protein synthesis (Sydiskis and Roizman, 1966; Powell and Courtney, 1975; Fenwick and Walker, 1978; Nishioka and Silverstein, 1978a) involving disaggregation of host polyribosomes (Sydiskis and Roizman, 1966, 1967; Fenwick and Walker, 1978; Nishioka and Silverstein, 1978b) and inhibition of host DNA and RNA synthesis (Roizman et al., 1965; Stenberg and Pizer, 1982). However, it has been shown that a specific set of cellular genes which encode polypeptides known as "stress" or "heat shock" proteins are activated in response to alterations, such as HSV-infection, in the cellular environment (Notarianni and Preston, 1982; LaThangue et al., 1984). In addition, Everett (1985) showed that activation of transcription of the rabbit beta-globin gene and the human epsilon-globin promoter linked to the HSV-thymidine kinase (TK) coding region, stably transfected into BHK thymidine kinase negative (TK^-) cells, is stimulated upon HSV-1 infection. These genes may be integrated into sites in the cellular genome which are particularly accessible to HSV-IE gene product activation.

Studies on the mechanism by which host macromolecular synthesis is shut off have shown that UV-irradiation of HSV-2 does not affect inhibition, suggesting that inhibition is due to a structural component of the virion. In addition, Isom et al. (1983) has shown that shut-off of host protein synthesis in hepatoma cells is mediated by a function requiring viral gene expression. Some strains of HSV-2, for example, strain G, shut off host protein synthesis more rapidly and efficiently than do HSV-1 strains (e.g. strain F) (Fenwick et al., 1979). This appears to be a general observation. However, the HSV-2 strain used in these studies, HG52, is less efficient as can be seen in several gels in this thesis. The basis for

the difference is not known, however the genetic locus responsible for shut-off by strain HG52 has been mapped by use of HSV-1 x HSV-2 intertypic recombinants to overlap that by strain G obtained by Morse et al. (1978) and Fenwick et al. (1979), 0.52-0.59 map units (mu) (R.G. Hope and H.S. Marsden, unpublished observations). Nishioka and Silverstein (1978b) reported that two distinct shut-off functions exist in HSV-1. One is a virion component, causing disaggregation of host polyribosomes and the other, requiring viral genome expression, causes degradation of host mRNAs and therefore shut-off of host protein synthesis.

The pathway by which virus is assembled in the nucleus is poorly understood. EM studies of infected cells show condensation and margination of host chromatin followed by the appearance of capsids (Morgan et al., 1959; Nii et al., 1968a; Miyamoto and Morgan, 1971; Smith and deHarven, 1973). Three temperature sensitive (ts) mutant-triggered features not previously described in HSV-1-infected cells were recognised by Dargan and Subak-Sharpe (1983): a modification of rough endoplasmic reticulum (ER), intranuclear accumulation of enveloped virus particles and cytoplasmic accumulation of novel doughnut-shaped particles having a concentric double-ring appearance in thin sections. By comparing the appearance of these structures and those previously observed by other workers (Morgan et al., 1968; Nii et al., 1968a, b, c; Schwartz and Roizman, 1969; Nii, 1971a, b) with the mutant block-time, the authors postulated that the block causes the overproduction of marginated chromatin, nuclear membrane distortion, intranuclear granular accumulations, nucleocapsid-related structures, nuclear membrane reduplication, modified ER, cytoplasmic membrane reduplication and the appearance of electron-dense cored virus particles. Furthermore, these observations probably reflect different stages in the normal HSV-replicative cycle. Other investigations examining the assembly of HSV virions have

included the use of HSV-2 ts mutants (Cabral and Schaffer, 1976; Atkinson et al., 1978) and metabolic inhibitors such as hydroxyurea (Nii et al., 1968b; Friedman et al., 1975) and cytochalasin B (Marciano-Cabral et al., 1977). EM studies have shown that HSV-2 produces tubular structures in the nuclei of infected cells (Murphy et al., 1967). Kinetic studies using metabolic inhibitors suggest that these tubular structures are intermediates in virus assembly rather than the result of aberrant capsid formation (Iwasaka et al., 1979; Oda et al., 1979).

It is not known how DNA is packaged into virions. Stow et al. (1983) has demonstrated that the a sequence, present at the ends of the terminal repeat regions and in inverted orientation at the L-S joint, is involved. Pignatti and Cassai (1980) isolated nucleoprotein complexes which included non-nucleosomal DNA with capsid-like structures at one terminus. However, it is not clear whether these structures represent true intermediates in the encapsidation process or break-down products.

Once the DNA is packaged, this nucleocapsid undergoes envelopment at the inner nuclear membrane, the virion is then transported from the perinuclear space to the outside of the cell. Schwartz and Roizman (1969) proposed on the basis of EM studies that the egress of virions from the cell was via a network of tubules thought to be formed in infected cells connecting the perinuclear region with the extracellular space. However, more likely, egress of virions is via a reverse phagocytosis (Morgan et al., 1959), that is, envelopment occurs at the inner nuclear membrane and egress is via transport vesicles operating between the rough ER and the golgi and between the golgi and the cell surface. Evidence supporting the reverse phagocytosis proposal comes from Tartakoff and Vassalli (1977, 1978) and Uchida et al. (1979) using monensin, an ionophore known to block the transport of proteins from the Golgi apparatus to the cell surface, which also blocked the transport of HSV

virions to the cell surface causing the accumulation of infectious virions in abnormally large cytoplasmic vacuoles (Johnson and Spear, 1982).

It is possible a mechanism exists which prevents the adsorption of progeny virus to the cells from which they have been released. Dissemination of infection could have a selective advantage if such a mechanism existed. Mapping studies (Tognon et al., 1981) using an HSV-1 non-lethal mutant indicates that a function encoded in the S component of the genome might prevent entry of progeny virus into the infected cell. Virus spreads from cell to cell via the medium and also by direct cell to cell passage (Stoker, 1958). While virtually all clinical isolates of HSV cause rounding of cells after infection in tissue culture some strains, after passage in tissue culture, produce cell fusion or syncytia. The cell fusion process is genetically determined and is discussed in more detail in Section 1.22.4.

1.5.2 Latent infection by HSV

A characteristic feature of herpesviruses is their ability to establish latency after a primary infection. The virus may then periodically reactivate from its latent state at the same peripheral site. The recurrences are often precipitated by a veriety of stimuli, e.g. excess exposure to sunlight (for reviews, see Stevens, 1975; Marsden, 1980; Wildy et al., 1982; Hill, 1985).

The presence of HSV-2 DNA in latently infected mouse ganglia was demonstrated by hybridisation techniques (Puga et al., 1978). HSV has been reactivated from explanted ganglia cultured in vitro (Stevens and Cook, 1971; Stevens, 1975; Warren et al., 1977; Al-Saadi et al., 1983; Tullo et al., 1983). Stevens and Cook (1971) were unable to detect viral antigens in latently infected mouse ganglia by immunofluorescence. Using a general antiserum to HSV, Green et al. (1981a) were similarly

unsuccessful with latently infected ganglia from rabbits. However, by using a monospecific antiserum, Green et al. (1981b) demonstrated the presence of the immediate-early polypeptide ICP4 ($V_{MW}175$) in the nuclei of ganglionic neurons.

HSV ts mutants have been used to investigate the mechanism of latency in the mouse (Lofgren et al., 1977; Watson et al., 1980; Clements and Subak-Sharpe, 1983; Al-Saadi et al., 1983). These studies showed that expression of the IE $V_{MW}175$ gene and at least one late gene was necessary for latency. By use of TK$^-$ mutants of HSV, it was shown that expression of the TK gene is necessary for efficient establishment of latency (Tenser and Dunstan, 1979; Tenser et al., 1979).

1.6 Herpesvirus DNA replication

Both pseudorabies virus and herpes simplex virus have been extensively used to investigate the replication of herpesvirus DNA. Only a small proportion of the HSV genomes appear to enter the replicative pool (Jacob and Roizman, 1977). The viral DNA is replicated semi-conservatively in the nucleus of the infected cells (Newton and Stoker, 1958; Munk and Sauer, 1964; Kaplan and Ben-Porat, 1964). HSV DNA synthesis in BHK 21 cells begins at about 2h post-infection (PI) and increases in rate until about 9-10h PI (Wilkie, 1973). Virus encoded enzymes known, so far, to be involved in viral DNA replication include DNA polymerase, ribonucleotide reductase, alkaline deoxyribonuclease and the major DNA-binding protein (see Section 1.8.4). Replication begins at a number of sites, around the periphery of the nucleus, which eventually fuse to occupy the entire nucleus (Rixon et al., 1983)

The precise mode of replication remains unclear. Viral DNA is thought to circularize following infection, since circular DNA molecules of unit length have been identified by EM (Schlomai et al., 1976; Rixon,

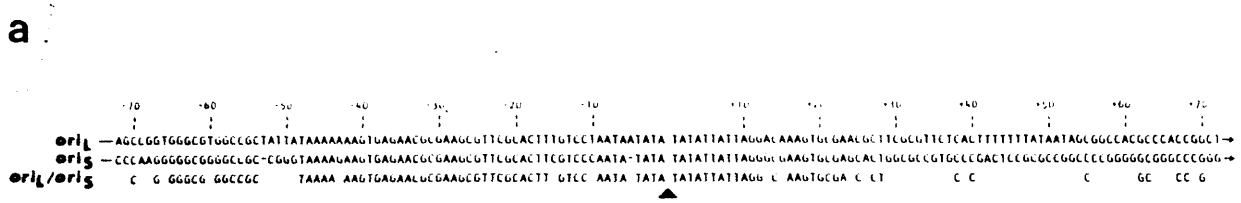
1977; Friedman et al., 1977; Hirsch et al., 1977; Jacob and Roizman, 1977; Ben-Porat and Veach, 1980) and thereafter to replicate, perhaps by a rolling circle mechanism (Jacob et al., 1979). Restriction endonuclease cleavage analyses show that terminal DNA fragments are less frequently generated from intracellular DNA than from virion DNA, which suggests that the molecule is "endless", probably in the form of head to tail concatemers (Jean and Ben-Porat, 1976; Jean et al., 1977; Ben-Porat and Tokuzewski, 1977; Ben-Porat and Rixon, 1977; Jacob et al., 1979).

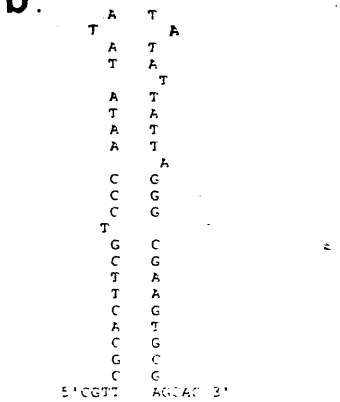
Two different origins of replication have been identified in HSV-1 DNA. One, designated ORI_L, is located within co-ordinates 0.407 and 0.429mu (Spaete and Frenkel, 1982). The other, designated ORI_S, is present in duplicate, one in each of TR_S and IR_S. Both ORI_S and ORI_L have been located in the HSV-1 genome, ORI_S by deletion analysis of HSV-1 DNA fragments cloned into a plasmid vector (Stow, 1982; Stow and McMonagle, 1983; Mocarski and Roizman, 1982a) and ORI_L by sequencing purified fragment BamHI U from class II defective HSV-DNA (Gray and Kaerner, 1984) and by cloning of HSV-DNA sequences between co-ordinates 0.398 and 0.413mu into a yeast cloning vector (Weller et al., 1985).

Stow and McMonagle (1983) have shown that the cis-acting sequences required for DNA replication lay within a 90bp region which contains a 45bp long palindromic sequence that can form a hairpin structure (fig. 2). Similarly, Weller et al. (1985) have shown that sequences required for DNA replication lay within a 425bp fragment which contained a perfect 144bp palindromic sequence with striking homology to ORI_S (fig. 2).

1.7 Transcription of HSV-DNA

Viral DNA is transcribed in the nucleus of infected cells by the host cell RNA polymerase II. This was demonstrated by the observation


a.
oriL
oriS
oriL/oriS


b.

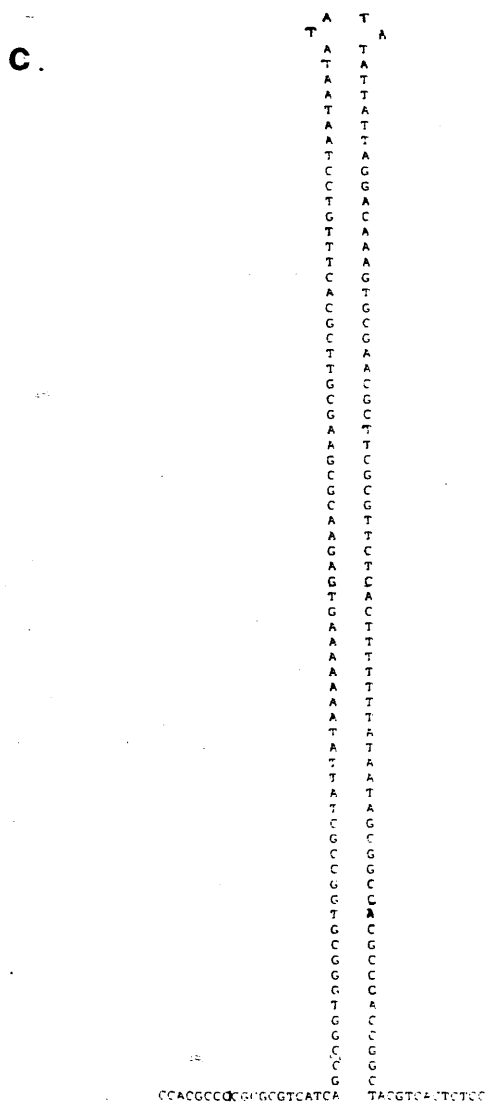

c.

FIGURE 2

Comparison of sequences comprising the origins of replication (ORI) in the long (L) and short (S) components of the HSV-1 genome.

(a) The sequences in ORI_L and the sequences in ORI_S are shown on the top and middle lines, respectively. Shared bases are shown on the bottom line. The centre of symmetry (▲) is indicated.

(b) is a representation of the palindrome formed by the sequences at ORI_S.

(c) is a representation of the palindrome formed by the sequences at ORI_L.

that HSV-transcription is sensitive throughout infection to alpha-amanitin, a specific inhibitor of eukaryotic RNA polymerase II (Alwine et al., 1974; Ben-Zeev et al., 1976). It is not known whether RNA polymerase II is modified during infection. Naked HSV-DNA is infectious, indicating that unmodified RNA polymerase II can transcribe the viral genome (Graham et al., 1973), however, it has been reported that RNA polymerase activity in infected cells and uninfected cells differs with respect to optimal conditions and reaction kinetics (Ben-Zeev et al., 1976) suggesting that it may indeed be modified.

Once transcription has occurred, the viral mRNA appears in the cytoplasm about 10-15min. later, where it becomes associated with polysomes (Wagner and Roizman, 1969b). The viral transcripts undergo post-transcriptional modifications, in common with most eukaryotic mRNAs. They are capped and methylated at their 5' ends (Bartoski and Roizman, 1976; Moss et al., 1977). Internal methylation has also been reported (Moss et al., 1977). The cap-site accurately identifies the transcription initiation site. The 3' ends of viral mRNA are polyadenylated (Bachenheimer and Roizman, 1972), in common with most eukaryotic mRNAs. The poly A tails vary in length from 30-200 bases (Silverstein et al., 1973, 1976). The polyadenylation signal AATAAA (Proudfoot and Brownlee, 1976) is located about 20-30bp upstream from the 3' end. A GT rich sequence located about 30bp downstream of the AATAAA signal is also thought to play a role in post-transcriptional processing of mRNA (McLauchlan et al., 1985).

Splicing has been noted in three IE and two late HSV-1 mRNAs to date. These are, the mRNAs of the IE genes, IE1 (Perry, Rixon, Everett, Frame and McGeoch, manuscript in preparation), IE4 and IE5 (Watson et al., 1981; Rixon and Clements, 1982), the mRNA family transcribed from the glycoprotein C gene (Frink et al., 1981, 1983) and a 2.7kb HSV-1

mRNA mapping between 0.185 and 0.225mu which could encode a protein with an approximate size of 75000 daltons (Costa et al., 1985). The introns (sequences of mRNA which are spliced out) conform to the GT---- AG rule for junctional sequences (Breathnach et al., 1981).

1.7.1 Temporal regulation of HSV-transcription

HSV-transcription shows temporal regulation and the mRNAs have been divided into three broad classes, immediate-early (IE or alpha), early (beta) or late (gamma), based on both their kinetics and their requirement for DNA synthesis (Frenkel and Roizman, 1972; Swanstrom and Wagner, 1974; Clements et al., 1977; Jones and Roizman, 1979). The IE class does not require de novo infected cell protein synthesis. The early class requires prior synthesis of IE polypeptides, but transcription can take place in the absence of DNA synthesis. The late mRNAs can be subclassified according to whether they can be detected prior to DNA synthesis (beta-gamma) or only after it (gamma) (Holland et al., 1980; see review by Wagner, 1985). The existence of at least four temporal transcription classes was apparent from studies with ts mutants of HSV-1 (Watson and Clements, 1978) and inhibitors of protein synthesis and DNA replication (Clements et al., 1977, 1979; Anderson et al., 1980).

The genome locations of the IE genes are shown in fig. 3. Transcription of these genes is positively regulated by a component in the virion (Post et al., 1981; Mackem and Roizman, 1982a; Cordingley et al., 1983; Batterson and Roizman, 1983). The virion component was identified as $V_{MW}65$ (Campbell et al., 1984). At least three IE mRNAs (those coding for $V_{MW}175$, $V_{MW}110$ and $V_{MW}68$) continue to be synthesised till late after infection (Anderson et al., 1980). The synthesis of the polypeptide product of IE mRNA 3 ($V_{MW}175$) is required continuously throughout infection for the synthesis of early and late viral RNAs (Watson and

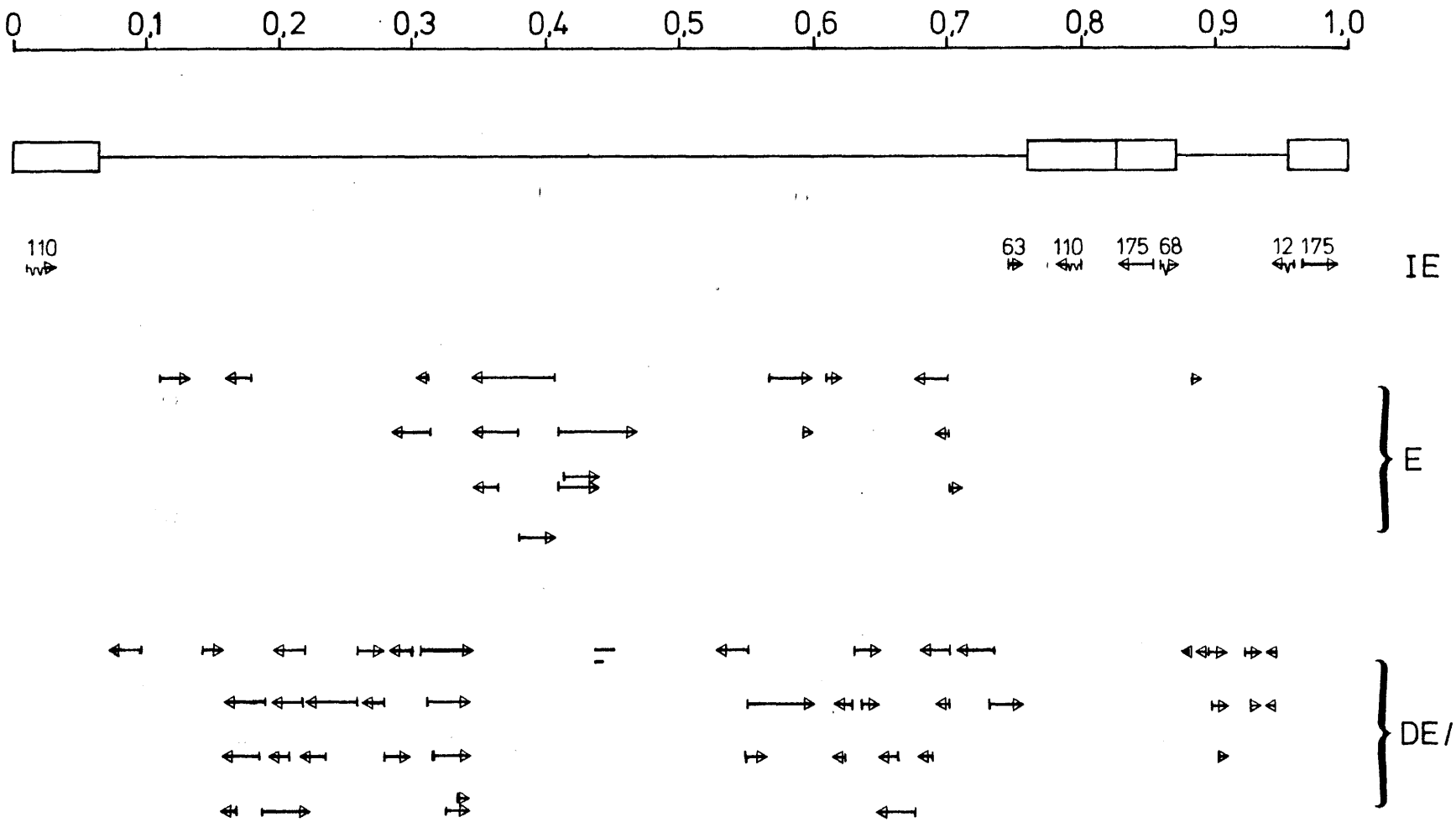

0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
1,0
110
63
110
175
68
12
175
IE
E
DE/L

FIGURE 3

Genomic locations of the known HSV-1 mRNAs. The data is taken from Wagner (1985) and Holland et al. (1984a). The top line represents the fractional genome length. The line below represents the HSV-1 genome in its prototype orientation as described in fig. 1. The arrows represent genomic locations and orientations of the immediate early (IE), early (E), and delayed early (DE) or late (L) mRNAs of HSV-1 and HSV-2. Above the arrows representing the IE mRNAs is shown the apparent MW ($x10^{-3}$), estimated from SDS-PAGE of the translation product of that mRNA.

Clements, 1980). The IE genes have a consensus sequence, TAATGARAT, as an upstream element 5′ to their promoter region. The consensus sequence is thought to act in the co-ordinate expression of these genes (Whitton and Clements, 1984; Preston et al., 1984a). Fig. 4 is a representation of the general structure of an HSV gene.

Studies with ts mutants have demonstrated that the IE polypeptide $V_{MW}175$ is required to promote transcription of E mRNAs (Preston, 1979a; Watson and Clements, 1978, 1980; Dixon and Schaffer, 1980). Everett (1984b) presented evidence showing that only the IE polypeptide $V_{MW}175$ on its own activates transcription of early genes, but that a combination of the IE polypeptides, $V_{MW}175$ and $V_{MW}110$, is a much better activator than $V_{MW}175$ on its own. More recently, O'Hare and Hayward (1985) and Quinlan and Knipe (1985) constructed plasmids which demonstrate that the $V_{MW}175$ and the $V_{MW}110$ polypeptides each possess the ability to stimulate expression of early genes. This apparent conflict of results may be a consequence of the systems used by the authors who each examined the activation of different HSV early gene promoters. Everett (1984b) suggested that viral IE gene products might interact with sequences involved in general promoter activity such as the TATA box. Since such sequences are not identical in all promoters, a given IE gene product could activate different promoters to different extents.

The approximate locations of at least twenty early HSV-1 mRNAs are known (Holland et al., 1979, 1980, 1984a) and, at late times, about forty HSV-1 mRNAs can be detected (Anderson et al., 1979; reviewed by Wagner, 1985) (fig. 3). However, the polypeptide products and the genome locations have been rigorously determined for only a few species. Recently, Rixon and McGeoch (1985) have analysed the mRNAs which map within the U_S component of the HSV-1 genome. Analysis by Northern blotting defines thirteen mRNA species, eleven of which are arranged into

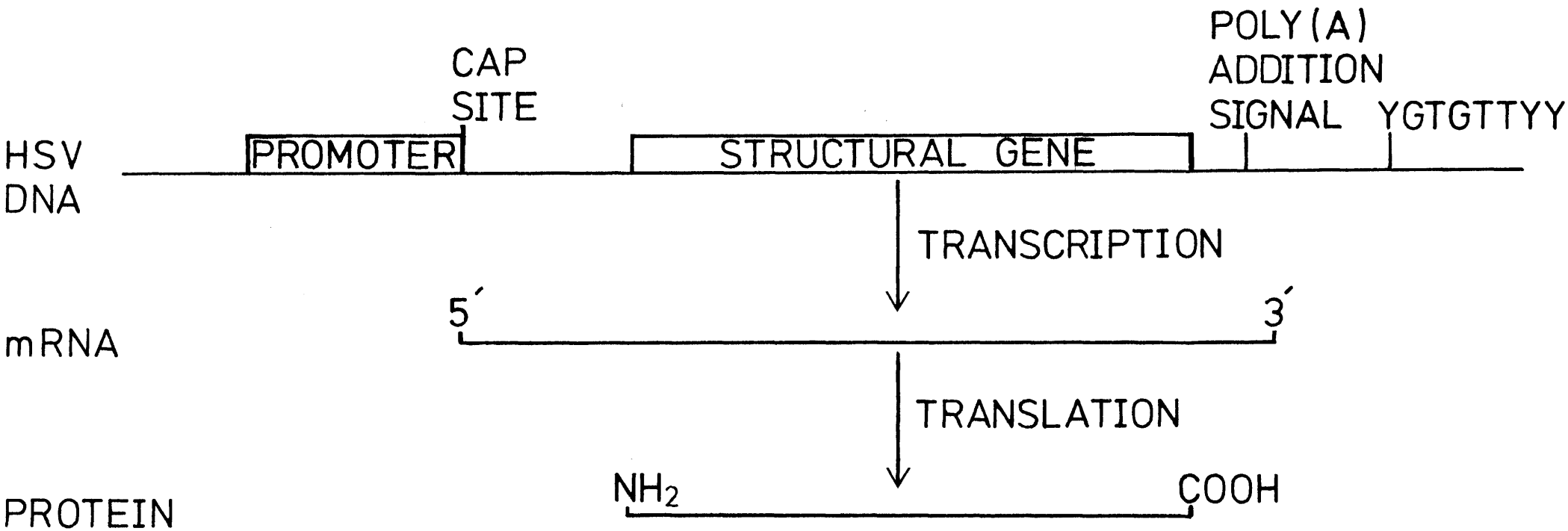

POLY(A)
ADDITION
SIGNAL
YGTGTTYY
CAP
SITE
HSV
DNA
PROMOTER
STRUCTURAL GENE
TRANSCRIPTION
5´
3´
mRNA
TRANSLATION
NH2
COOH
PROTEIN

FIGURE 4

Representation of a general structure for an HSV-encoded early (beta) or late (gamma) gene. Each structural gene has its own promoter which contains sequences essential for initiation and control of levels of mRNA. For gD-1 these sequences extend 83bp upstream of the mRNA start site and include a TATA box (Breathnach and Chambon, 1981; Benoist et al., 1980; Efstratiadis et al., 1980), usually located about 20bp upstream of the mRNA start site, and may include G-rich and AC-rich sequences thought to be important for the activation of transcription (Everett, 1983). The polyadenylation signal is located about 20bp before the end of the mRNA. The consensus sequence YGTGTTYY (where Y = pyrimidine) is thought to play a role in post-transcriptional processing of the 3' terminus.

four nested families. Previously, the DNA sequence of the U_S component of the HSV-1 genome had been determined (McGeoch et al., 1985) from which twelve genes were predicted, six of which were previously unknown. Hybridisation experiments enabled each of the thirteen mRNAs to be assigned to the twelve predicted genes in U_S (fig. 5).

This is the first description of the transcription pattern of an extensive region of the HSV-1 genome for which the complete DNA sequence is known. Some of the individual mRNAs had been mapped previously. The mRNAs for gene US1 and gene US12 (encoding IE68 and IE12, respectively) were mapped by Clements et al. (1979) and Anderson et al. (1980), the mRNA for gene US6 (gD) was mapped by Lee et al. (1982b) and Watson et al. (1982) and the mRNAs for genes US10 (21K) and US11 (33K) were mapped by Lee et al. (1982b). Two other mRNAs mapping in the short region were identified by Lee et al. (1982b), one specified translation of a 42K polypeptide, maps to the left of gD and may be the translation product of gene US4, the other mRNA specified translation of a 55K polypeptide, co-maps with gD and may be the translation product of gene US7.

Four mechanisms have been described whereby nested "families" of HSV-mRNAs can be generated: (1) secondary promoters close to each other, (2) interior promotion within the sequences encoding another mRNA, (3) the use of alternative poly A sites, and (4) splicing (for review, see Wagner, 1985). The transcript formed by one of these mechanisms, encode proteins which have been shown to be functionally related. Two mRNAs which share 3' termini are transcribed from the genes encoding the $V_{MW}136'$ (143) and $V_{MW}38$ polypeptides (McLauchlan and Clements, 1982, 1983). These two polypeptides have been shown to be involved in the HSV-1 ribonucleotide reductase activity: the larger one being an essential constituent of the enzyme (Dutia, 1983; Preston et al.,

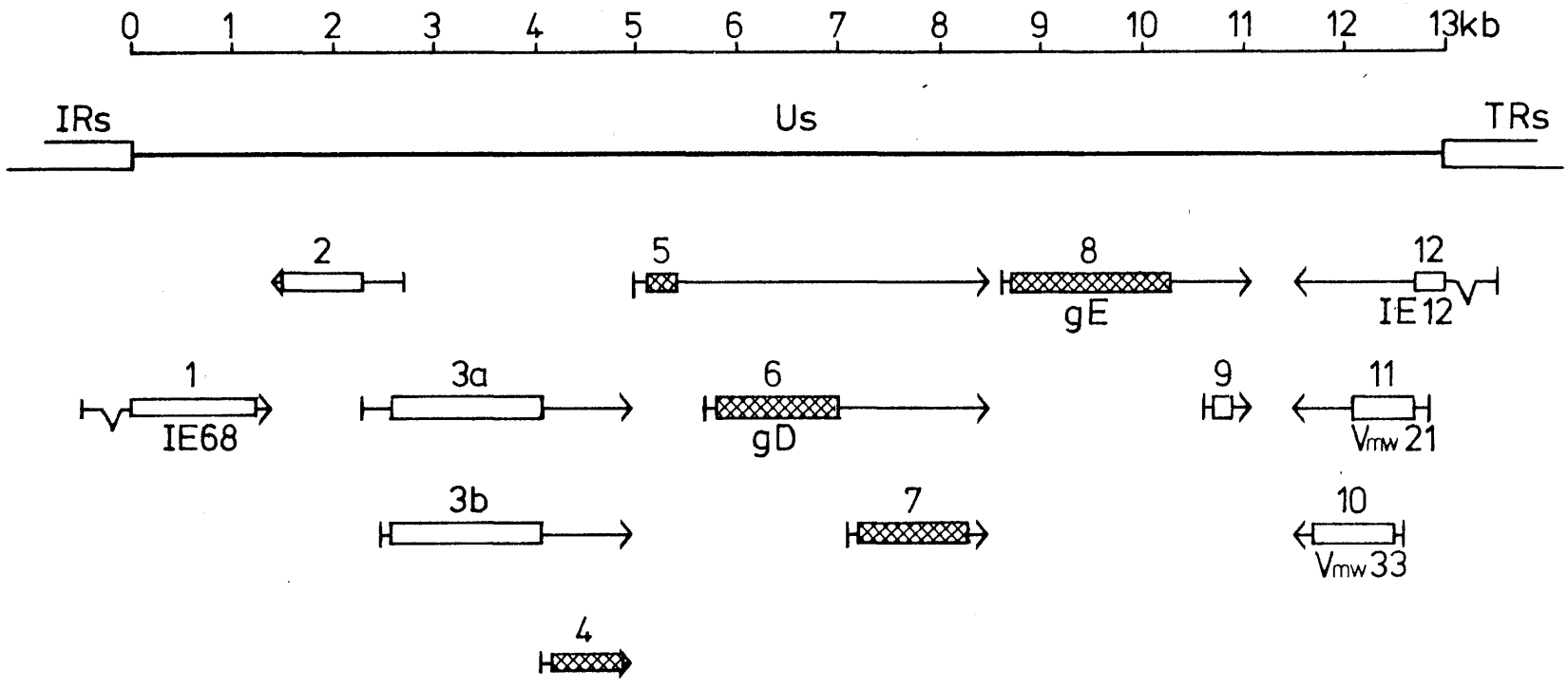

0 1 2 3 4 5 6 7 8 9 10 11 12 13kb
IRs
Us
TRs
2
5
8
gE
12
IE12
1
IE68
3a
6
gD
9
11
Vmw21
3b
7
10
Vmw33
4

FIGURE 5

Summary of the mRNA mapping data of the short region of the HSV-1 genome (from Rixon and McGeoch, 1985). The short unique component (U_S) and part of the adjacent inverted repeat regions of the HSV-1 genome is represented. The scale is in kilobases (kb) from 1 at the IR_S/U_S junction to 13kb just inside TR_S. The numbers identify the 12 genes predicted by analysis of DNA sequence data (McGeoch et al., 1985). The boxes represent the coding region of these genes. Those boxes which are cross-hatched are predicted to be glycoproteins. The arrow represents the map location and direction of transcription of the mRNAs. The protein designation, where known, is indicated below the mRNA, for example, from mapping data discussed elsewhere in the text, it is believed that gene 8 encodes gE and gene 11 encodes the $V_{MW}21$ protein which binds to the a sequence (Dalziel and Marsden, 1984).

1984b) and the smaller one forming a complex with the larger (Frame et al., 1985). This observation raises the possibility that the translation products within some other nested families of mRNAs described by Rixon and McGeoch (1985) may also be functionally related.

1.8 HSV protein synthesis

1.8.1 Regulation of synthesis of HSV-induced proteins

Honess and Roizman (1974) made use of metabolic inhibitors to show that the synthesis of HSV-induced polypeptides is temporally regulated. The authors proposed a cascade regulation of synthesis whereby the virus-specific proteins can be divided into three groups termed alpha (immediate-early, IE), beta (early) or gamma (late) by the criteria of maximum rate of synthesis and dependence on prior viral protein or DNA synthesis.

The IE polypeptides are synthesised without prior viral protein or DNA synthesis and are synthesised maximally between 2h and 4h PI. Five major IE polypeptides have been identified in HSV-1 infected cells, $V_{MW}175$, $V_{MW}110$, $V_{MW}68$ and $V_{MW}63$ (Honess and Roizman, 1974; Pereira et al., 1977; Preston et al., 1978; Fenwick et al., 1980) and $V_{MW}12$ (Watson et al., 1979; Marsden et al., 1982).

In the original scheme proposed by Honess and Roizman (1974), the synthesis of early polypeptides requires prior synthesis of IE polypeptides and new RNA synthesis, early polypeptides are synthesised at maximal rates 5-7h PI and at decreasing rates thereafter. These authors suggested that the early polypeptides are involved in reducing the synthesis of IE polypeptides and also in the regulation of late polypeptides. By examining the effect of ts mutations in the gene encoding the early polypeptide $V_{MW}136'$ (130) which is the major DNA binding protein, Godowski and Knipe (1983, 1985) showed that this polypeptide is involved in these

functions.

Late polypeptides are synthesised at increasing rates until at least 12h PI and their synthesis requires prior synthesis of early polypeptides and viral DNA synthesis. Honess and Roizman (1974) suggested that the late polypeptides reduce the synthesis of early polypeptides.

The classification is not entirely satisfactory since some proteins in the early class are expressed under IE conditions in HSV-infected human foetal lung (HFL) cells (McDonald, 1980). Also, other proteins, e.g. gB-1 and gD-1 do not fit neatly into the classification scheme of Honess and Roizman (1974). gB-1 and gD-1, like early polypeptides, are synthesised during intermediate stages of the replicative cycle and can be expressed in the absence of viral DNA synthesis, but like true late polypeptides, are synthesised at maximal rates only after DNA synthesis (Gibson and Spear, 1983). Such polypeptides have been termed beta-gamma, early-late or delayed-early (Wagner, 1985). Data by Marsden et al. (1976) from analysis of the polypeptides produced by sixteen ts mutants of HSV-1 provided further information as to the control of virus polypeptide synthesis. These authors showed that the virus-induced polypeptides can be grouped into nine classes based upon the requirement of each polypeptide for DNA synthesis by each of the mutants.

1.8.2 Number of HSV-encoded proteins

More than fifty HSV-1-induced polypeptides have been detected by one-dimensional SDS-PAGE (Honess and Roizman, 1973; Powell and Courtney, 1975; Marsden et al., 1976). Analysis of purified virions has identified 33 of these polypeptides as structural (Heine et al., 1974; Marsden et al., 1976). Haarr and Marsden (1981) have used the technique of two-dimensional (2-D)-PAGE to show 230 virus-induced polypeptides present in HSV-1-infected cells. Many of these polypeptides are related

through post-translational modifications, for example, at least twelve of the polypeptides are intermediates in the synthesis of gD, however, it is likely that at least some of the polypeptides only resolved by 2-D-PAGE will represent new gene products.

Investigators have used a variety of methods to establish that the proteins synthesised by HSV-1 and HSV-2 are functionally comparable. These methods include complementation between ts mutants of HSV-1 and HSV-2 (Timbury and Subak-Sharpe, 1973), isolation of intertypic recombinants (Timbury and Subak-Sharpe, 1973; Wilkie et al., 1977; Morse et al., 1977; Preston et al., 1978), intertypic marker rescue (Stow, 1978) and cross-neutralisation tests (Killington et al., 1977).

It is likely that the number of known HSV-encoded polypeptides will increase since studies on the sequence of the HSV genome will help to predict polypeptides which were previously undetected because of their low abundance in the infected cell.

(a) Genomic location of HSV-induced polypeptides

The polypeptide profiles on SDS-polyacrylamide gels of HSV-1- and HSV-2-infected cells are very similar, but not identical (Cassai et al., 1975; Killington et al., 1977). The ability to recognise a given polypeptide as being of type 1 or type 2 and the analysis of intertypic recombinants has enabled many HSV-induced polypeptides to be mapped onto the genome (Marsden et al., 1978, 1984; Morse et al., 1978; Ruyechan et al., 1979). Restriction enzymes were used to determine from which parental serotype (HSV-1 or HSV-2) each region of the recombinant genome originated. Other techniques used to locate genes encoding HSV-induced polypeptides include in vitro translation of selected mRNAs (Docherty et al., 1981; Lee et al., 1982b) and hybrid-arrest of translation (Preston and McGeoch, 1981; Docherty et al., 1981). Extensive sequencing

of the HSV-genome is currently underway and has located many genes precisely, including some which were previously unidentified (see Section 1.7.1). Fig. 6 is a summary of the mapping data for those proteins which have not been described elsewhere in this thesis (see figs. 3, 5, 7 and 17).

1.8.3 Post-translational modifications

At least four types of post-translational modifications are known to occur in HSV-induced polypeptides. These are glycosylation and sulphation which will be discussed in detail in Chapter 1, Section C, and phosphorylation and cleavage.

(a) Phosphorylation

There are 16 HSV-1 and 18 HSV-2 phosphorylated polypeptides which can be detected in infected cells (Marsden et al., 1978; Pereira et al., 1977; Wilcox et al., 1980). The identified phosphorylated polypeptides are listed in Table 2. The map locations of some of them are indicated in fig. 6 and fig. 7. Wilcox et al. (1980) demonstrated that phosphate cycles on and off some HSV-1-induced polypeptides, ICP4 (IE175), ICP6 ($V_{MW}136'$ (143)), ICP22 (IE68) and ICP27 (IE63) and that it can alter the affinity of HSV-1 $V_{MW}136'$ (143) and HSV-2 $V_{MW}57'$ (57) for DNA. Wilcox et al. (1980) also observed IE175 was phosphorylated during a pulse label, whereas IE68 and IE63 were phosphorylated only after a subsequent chase period. The authors concluded that IE68 and IE63 were phosphorylated either by a different viral or cellular phosphoprotein acting as a phosphate donor, other than that which phosphorylates IE175, or by using phosphate from different pools.

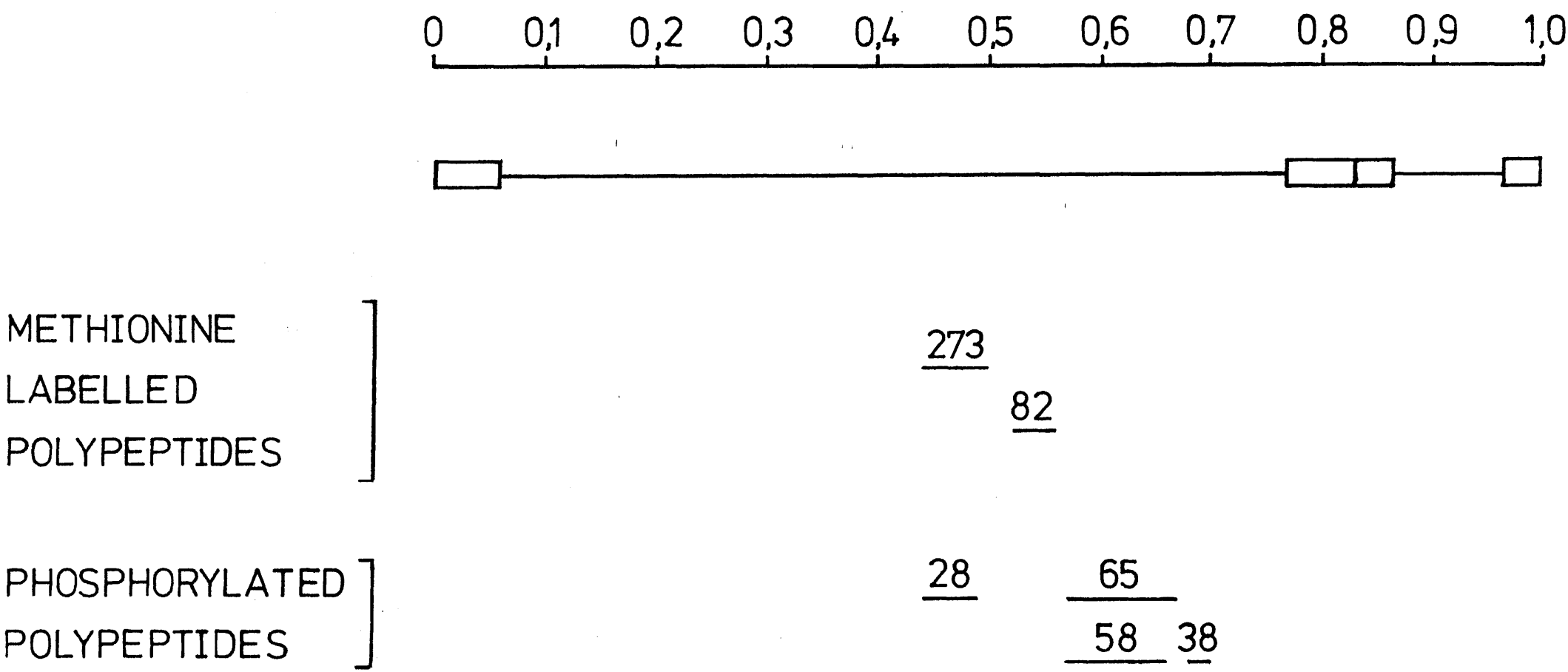

0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
0,9
1,0
METHIONINE LABELLED POLYPEPTIDES
273
82
PHOSPHORYLATED POLYPEPTIDES
28
65
58
38

FIGURE 6

Genome locations of HSV-polypeptides which are not dealt with elsewhere in this study. The scale at the top represents the fractional genome length (mu). Below the scale is a representation of the HSV genome, long (L) and short (S) components are indicated, in the prototype configuration. The shorter horizontal lines represent the physical map locations of some polypeptides. The numbers above the lines are the apparent MW ($x10^{-3}$) estimated from SDS-PAGE (Marsden et al., 1978). The map locations of the genes encoding other viral polypeptides are indicated as follows:

IE polypeptides - fig. 3; methionine-labelled polypeptides including regulatory and structural proteins and enzymes - fig. 7; phosphorylated polypeptides (table 2) either have enzymic functions (see Section 1.8.4) or are IE polypeptides (see fig. 3); glycoproteins - figs. 5 and 17.

TABLE 2

HSV-1		HSV-2	
V_{MW}[a]	ICP[b]	V_{MW}	ICP[b]
175	4	182	4
136'(143)	6	138'(144)	6
117	11	118	11
87		90	
85	19	84	19
82		82	
68	22	67	22
65'(65)	25	65'(66)	25
65'(55)		65'(64)	26
63	27	65'(61)	27
58		57'(57)	29[c]
		57'(56)	
45		38	
38'		36'	41
36		35	
32		32	
28	44	29.5	
21		20	

a apparent MW ($x10^{-3}$), Marsden et al. (1978)

b infected cell polypeptide, Wilcox et al. (1980)

c ICP29 is split into two bands in some gels

TABLE 2

Comparison of nomenclature for some HSV-1 and HSV-2 phosphorylated polypeptides. The comparison is based on protein profiles of ^{32}P-labelled HSV-infected cells resolved on 9% SDS-polyacrylamide gels cross-linked with diallyltartardiamide. It should be noted that labelling times used by each author were different, therefore, some phosphorylated polypeptides are observed by one set of authors, but not by the other. Also, the strain of virus used and the type of cells in which the virus is grown may affect the degree of phosphorylation.

(b) Cleavage

A number of HSV-encoded proteins undergo post-translational cleavage. These include the large subunit of the ribonucleotide reductase complex $V_{MW}136'$ (143), which yields two polypeptides of apparent MW 100000 and 90000, $V_{MW}63$ which yields a 26000 polypeptide and IE175 and IE110, though the products of these could not be identified (McDonald, 1980). Breakdown was attributed to proteolytic cleavage since it did not occur in the presence of the proteolytic inhibitor tosylphenylchloromethyl ketone (TPCK). It is not known whether cleavage of these polypeptides has any biological significance. Proteolytic cleavage also occurs during processing of the HSV glycoproteins (see Section 1.20.5).

1.8.4 Functions of the HSV-induced polypeptides

HSV encodes a number of enzymes involved in its replication. Those enzymes which are encoded by the virus are: (1) alkaline deoxyribonuclease (Morrison and Keir, 1968), (2) deoxynucleoside pyrimidine kinase (TK) (Kit and Dubbs, 1963; Jamieson et al., 1974), (3) DNA polymerase (Keir and Gold, 1963), (4) ribonucleotide reductase (Cohen, 1972), (5) deoxyuridine triphosphate nucleotidylhydrolase (Caradonna and Cheng, 1981). A number of enzymes which, as yet, are only known to be virus-induced include (6) uracil DNA-glycosylase (Caradonna and Cheng, 1981), (7) cyclic AMP independent protein kinase (Blue and Stobbs, 1981) and (8) topoisomerase (Biswal et al., 1983).

Since some HSV-induced polypeptides bind to DNA, it might be expected that they have enzymic activity. Sixteen HSV-1 DNA binding proteins have been identified by Bayliss et al. (1975). For some of them, it is known what they do, for example, $V_{MW}85$ is the alkaline deoxyribonuclease (Section 1.8.4 (a)), $V_{MW}65$ is likely to be the topoisomerase (Section 1.8.4 (e)) and $V_{MW}43$ is the TK (Section 1.8.4 (b)).

Another subset of HSV-induced polypeptides which might be expected to have regulatory activity are the IE polypeptides. As already mentioned, two of these, IE175 and IE110, have been shown to stimulate transcription of early genes (Everett, 1984b; O'Hare and Hayward, 1985; Quinlan and Knipe, 1985). A summary of the mapping data of some of these enzymes and regulatory proteins is shown in fig. 7.

(a) Alkaline deoxyribonuclease

Cells infected with HSV exhibit an increase in activity of a deoxyribonuclease which has an alkaline pH optimum (Keir and Gold, 1963; Keir, 1968). Experiments with partially purified enzyme indicated that it had exonuclease activity specific for DNA (Morrison and Keir, 1968). Highly purified enzyme contained both exo- and endonuclease activity and to have an apparent MW of about 68000 (68K) (Hoffman and Cheng, 1978, 1979). The enzyme purified by Strobel-Fidler and Francke (1980) was composed of at least four polypeptides ranging from 70K to 90K. Moss et al. (1979) mapped the enzyme activity to between 0.12 and 0.21mu. In vitro translation studies showed that a major product of this area of the genome encodes an 85K polypeptide (Preston and Cordingley, 1982). More recently, it has been demonstrated by in vitro translation of selected mRNA that a 2.3kb mRNA which maps between 0.16 and 0.176mu encodes the exonuclease activity and that the mRNA encodes a polypeptide of 82K (Costa et al., 1983). Banks et al. (1983) confirmed that the $V_{MW}85$ polypeptide (ICSP22, ICP19) was responsible for the nuclease activity. The authors made use of monoclonal antibodies directed against the 85K polypeptide to neutralise enzymic activity. The enzyme activity is essential for viral-DNA replication (Moss et al., 1979).

IE110(ICP0)
alkaline exonuclease(Vmw85)
thymidine kinase (Vmw43)
DNA-encapsidation (p40)
major DNA binding protein (Vmw136'(130))
DNA polymerase (Vmw145)
ribonucleotide reductase (large subunit,Vmw136'(143)) (small subunit,Vmw38)
stimulation of E transcription (Vmw65)
dUTPase
IE63(ICP27)
stimulation of early transcription
IE110(ICP0)
IE175(ICP4)
IE68(ICP22)
21K
IE175(ICP4)
uracil-DNA glycosylase
cyclic AMP independent protein kinase
nucleoside phosphotransferase
topoisomerase

FIGURE 7

Genome locations of HSV-induced regulatory proteins and enzymes. The figure shows a representation of the HSV genome as described in fig. 6. The solid boxes represent that part of the genome encoding a protein, precise map co-ordinates can be found in the text. Those HSV ORF's written above the genome structure have been sequenced, those HSV ORF's written below the genome structure have not been sequenced and the enzymes named at the bottom of the figure have not yet been shown to be viral coded. All the proteins mapped onto the genome are phosphorylated except TK, DNA polymerase and the major DNA binding protein.

(b) Deoxynucleoside pyrimidine kinase (TK)

When TK$^-$ cells are infected with HSV, TK activity increases; this increase is due to a viral enzyme (Kit and Dubbs, 1963). The enzyme activity is induced at about 2h after infection and increases till 8h after infection. Compared with the host activity, the virus-specific enzyme has a low pH optimum, a low Km, is relatively insensitive to inhibition by thymidine triphosphate and is not rapidly inactivated by incubation at 40°C (Klemperer et al., 1967). The enzyme phosphorylates both thymidine and deoxycytidine using the same active site (Jamieson and Subak-Sharpe, 1974). The gene encoding TK activity maps between co-ordinates 0.300 and 0.309mu (Halliburton et al., 1980) and has been sequenced (McKnight, 1980; Wagner et al., 1981; D. McGeoch, unpublished data). It was originally reported that the active enzyme consists of a dimer of identical subunits, 42000 daltons each (Jamieson and Subak-Sharpe, 1974; Cheng and Ostrander, 1976).

Recently Preston and McGeoch (1981) showed that hybrid-arrested translation yielded two polypeptides of apparent MW 43K and 39K. Using the higher resolving ability of 2-D PAGE, three polypeptides are translated from a single mRNA using three in-phase initiation codons (Marsden et al., 1983; Haarr et al., 1985). The 39K polypeptide has activity since the HSV-1 strain Δ1 in which the first AUG codon is deleted, has TK activity (Haarr et al., 1985). It is not known, however, if the 38K polypeptide has TK activity.

(c) DNA polymerase

Keir and Gold (1963) detected an HSV-1-induced DNA polymerase activity in infected cells which differed from that of the host cell with respect to its heat stability, magnesium requirements and its ability to use different DNA primers. Keir et al. (1966b) suggested that the enzyme was

virus-specific since it was inhibited by antiserum to virus-infected cells. Similar results have been obtained for HSV-2 (Hay et al., 1971; Purifoy and Benyesh-Melnick, 1974, 1975). The DNA polymerase of HSV is sensitive to phosphonoacetic acid (PAA) (Mao et al., 1975; Leinbach et al., 1976 Hay and Subak-Sharpe (1976).

The HSV-2 DNA polymerase has been purified (Powell and Purifoy, 1977; Vaughan et al., 1985) and has an apparent MW estimated from SDS-PAGE to be about 150K. These authors observed that another polypeptide of apparent MW 54K co-purifies with the enzyme. These observations are consistent with those made by Powell and Purifoy (1977), Knopf (1979), Derse et al. (1982) who showed that the purified HSV-1 enzyme consisted of a major polypeptide of apparent MW 150K and at least one other polypeptide.

HSV-DNA polymerase has been shown to possess, in addition, a 3'-5' exonuclease activity (Knopf, 1979). Vaughan et al. (1985) investigated the function of the 54K polypeptide which co-purifies with the HSV-2 DNA polymerase but neither a monoclonal antibody directed against the 54K polypeptide nor a polyclonal HSV-2-specific antiserum neutralised the 3'-5' exonuclease activity. The authors also mapped the target antigen (54K) with a monoclonal antibody using an immunoperoxidase assay to between 0.525 and 0.647mu. These co-ordinates are distinct from the map position for DNA polymerase activity (0.400-0.418mu), which was mapped by intertypic marker rescue using ts mutants and PAA resistant (PAA^r) mutants (Chartrand et al., 1979, 1980). The region encoding the DNA polymerase of HSV-1 was located and the gene has been sequenced by Quinn (1984) and Gibbs et al. (1985). The predicted MW of the polymerase protein, 136,664, is compatible with the observed apparent MW on SDS gels.

(d) Ribonucleotide reductase

The HSV-induced ribonucleotide reductase activity differs from the host cell enzyme activity in a number of ways (Cohen, 1972). Concentrations of dTTP and dATP which completely inhibit the host enzyme activity, do not inhibit the viral enzyme activity and unlike the host enzyme, the viral enzyme has no absolute requirement for Mg^{++} (Ponce deLeon et al., 1977; Langelier et al., 1978; Huszar and Bacchetti, 1981).

Dutia (1983) made use of an HSV-1 ts mutant G to show that the enzyme is viral coded. She detected viral enzyme activity in cells infected with this mutant at the permissive temperature (PT), but not at the non-permissive temperature (NPT). Preston et al. (1984b) constructed an HSV-1 ts mutant, 17 ts VP1207 (ts 1207) by cloning the ts mutation of ts G into wild type HSV-1. The ts lesion in ts 1207 was found to map between co-ordinates 0.580 and 0.585mu within the structural gene for $V_{MW}136'$ (143). The lesion results in a thermolabile enzyme activity identifying $V_{MW}136'$ (143) as an essential component of the viral enzyme activity. It was also observed (Preston et al., 1984b; Bacchetti et al., 1984) that a 38K polypeptide co-purifies with the $V_{MW}136'$ (143) polypeptide. Similar observations were made for the HSV-2 enzyme activity (Bacchetti et al., 1984; Huszar and Bacchetti, 1981).

Recently, Frame et al. (1985) demonstrated that $V_{MW}136'$ (143) and $V_{MW}38$ form a complex in infected cells. They used the HSV-1 mutant ts 1207 and showed that a monoclonal antibody directed against the $V_{MW}136'$ (143) and an oligopeptide-induced antiserum directed against the carboxy-terminus of the $V_{MW}38$, immunoprecipitated both polypeptides at the PT, but only the polypeptide against which the antibodies were directed at the NPT. The authors concluded that $V_{MW}136'$ (143) and $V_{MW}38$ can form a complex and that the ts mutation in $V_{MW}136'$ (143) results in the complex

being unable to form at the NPT. They further speculated that complex formation was necessary for enzyme activity. The role of $V_{MW}38$ in enzyme activity remains to be demonstrated. They proposed the terms rr_1 and rr_2 for the large and small subunits, respectively.

(e) Topoisomerase

Topoisomerases relax supercoiled DNA by breaking the phosphodiester backbone of DNA then resealing the free DNA ends (for review, see Champoux, 1978; Gellert, 1981, Liu, 1983). Type I topoisomerases catalyse the breaking and rejoining of one strand of DNA at a time, while type II topoisomerases catalyse double-strand breaks (Gellert, 1981; Liu, 1983). The type I and type II enzymes can be distinguished by the requirement of type II, but not type I, for ATP and $MgCl_2$ (Marini et al., 1980).

Biswal et al. (1983) reported a topoisomerase activity which co-purified with HSV-induced DNA polymerase. These authors suggested that the topoisomerase activity is virus-induced, since extracts derived from cells infected at the PT, but not the NPT, with the early regulatory mutants of HSV-1, tsK and tsB2, contained activity. Leary and Francke (1984) presented evidence that HSV-induced a topoisomerase activity, as assayed by catenation of DNA. Muller et al. (1985) showed that the enzyme activity is associated with purified virions, being localised outside the nucleocapsid and that since the enzyme had no requirement for ATP or $MgCl_2$, it was a type I topoisomerase.

Topoisomerases are known to alter levels of transcription (North, 1985). The possibility that the 65K protein which stimulates transcription from IE genes (Campbell et al., 1984, see Section 1.7.1) and the co-migrating 65K DNA binding protein, DBP4 called 62K in the study of Bayliss et al. (1975) (see Section 1.8.4 (g)) were one and the same protein

and that the protein acted by having topoisomerase activity is currently under investigation in the laboratories of H.S. Marsden, C.M. Preston, D. Parris and M. Muller (manuscripts in preparation). Immunological and biochemical evidence was obtained to show that the two 65K proteins are different and that there is a close, but not proven, association between the 65K DNA binding protein and topoisomerase activity.

(f) Deoxyuridine triphosphate nucleotidylhydrolase (dUTPase)

The dUTPase catalyses the hydrolysis of dUTP to dUMP. As a consequence of this reaction, the enzyme minimises incorporation of dUTP into DNA and provides a supply of dUMP which can be converted into TMP by thymidylate synthetase.

After infection with HSV, the activity of dUTPase increases (Wohlrab and Francke, 1980; Caradonna and Cheng, 1981) and the new activity can be distinguished from that of the host by several criteria. First, the viral-induced enzyme, unlike the host enzyme, is active at +4°C (Caradonna and Cheng, 1981; Williams, 1984). Second, non-denaturing gel electrophoresis of extracts derived from HSV-1- and HSV-2-infected cells indicate that the virus-induced enzyme has a distinct electrophoretic mobility from that of the host enzyme. Third, serum from rabbits immunized against cells infected with HSV-1 or HSV-2, specifically neutralise the viral enzyme (Caradonna and Cheng, 1981). All these data strongly suggest that HSV encodes its own dUTPase.

The map location obtained for the gene encoding the HSV-induced dUTPase (Wohlrab et al., 1982) using HSV-1 x HSV-2 intertypic recombinants was later readjusted using accurately aligned HSV-1 and HSV-2 restriction endonuclease maps, to be compatible with that of 0.69 to 0.70mu obtained by Preston and Fisher (1984) who transfected cloned HSV-1 DNA fragments into BHK cells then assayed for enzyme activity. One

clone inducing virus-specific enzyme activity contained a gene specifying a 1.5kb mRNA which upon in vitro translation of selected mRNA, was shown to encode a 39000 MW polypeptide. It has subsequently been shown using both dUTPase deficient insertional mutants and a spontaneous dUTPase deficient mutant that the 39000 MW polypeptide is the virus coded dUTPase and that the dUTPase is not required for virus replication in tissue culture (Fisher and Preston, 1986).

(g) HSV-DNA binding proteins

Sixteen to eighteen virus-induced DNA-binding proteins can be detected in HSV-infected cells (Bayliss et al., 1975; Purifoy and Powell, 1976; Powell and Purifoy, 1976; Becker et al., 1980; Bookout and Levy, 1980; Hay and Hay, 1980; Wilcox et al., 1980). Of these, ICP4 ($V_{MW}175$) binds to DNA only via a component of mock-infected cells, since purified $V_{MW}175$ is unable to bind DNA, even though the protein is capable of such activity in the presence of an extract of uninfected cells (Freeman and Powell, 1982). Of the others, only one $V_{MW}136'$ (130) (ICP8, termed the major DNA-binding protein) has been purified sufficiently to show that it binds directly to DNA in the absence of other proteins.

The major DNA binding protein binds single-stranded DNA more strongly than double-stranded DNA (Bayliss et al., 1975; Powell and Purifoy, 1976). Powell et al. (1981) demonstrated that purified protein acted directly on a polydeoxyadenylic acid - polydeoxythymidylic acid helix, reducing its melting temperature, thus indicating that the protein functions in virus-DNA synthesis. The EM observations of Ruyechan (1983) that purified protein holds single-stranded DNA in an extended conformation, also suggests that the protein functions in virus-DNA synthesis. Evidence was presented by Littler et al. (1983), using ts mutants whose lesion was in the structural gene for the major DNA binding protein, that upon

shift-up to the NPT, viral DNA synthesis is switched off and that the HSV alkaline deoxyribonuclease and DNA polymerase activities are destabilized. Since these two enzymes also play a role in viral-DNA synthesis, the observations of Littler et al. (1983), support those of Powell et al. (1981) and Ruyechan (1983).

Godowski and Knipe (1983) suggested another function of the major DNA binding protein. The authors made use of ts mutants whose lesion resided in the structural gene for this protein (Lee and Knipe, 1983; Weller et al., 1983), to demonstrate that the synthesis of certain IE (IE175), early (V_{MW}136' (143), 136' (130) and 43) and late (V_{MW}155, 117, 100 and 65) polypeptides was greater in cells infected with the ts mutants at the NPT than in cells infected with wild-type virus or in cells infected with mutants at the PT. These results indicate that the protein acts as a negative regulator of viral gene expression. It seems pertinent to recognise that Honess and Roizman (1974, 1975) proposed that early proteins negatively regulate IE gene expression. The major DNA binding protein is the first example confirming this proposal.

The V_{MW}21 DNA-binding protein interacts specifically with the a sequence of HSV-DNA (Dalziel, 1984; Dalziel and Marsden, 1984). The properties and functions of the a sequence have been discussed in Section 1.4.1. Whilst there is no direct evidence concerning the role of the V_{MW}21 polypeptide, the authors proposed that it may be involved in packaging or circularisation of the genome (Dalziel and Marsden, 1984).

SECTION B: GLYCOPROTEIN STRUCTURE AND FUNCTION

1.9 Description

Glycoproteins are a ubiquitous class of proteins having carbohydrate moieties covalently attached to the peptide backbone. They can be found, for example, as hormones, e.g, thyroid stimulating hormone (TSH) (Carsten and Pierce, 1963), enzymes, e.g. pepsin (Bohak, 1969), immunoglobulins, e.g. IgG (Rosevear and Smith, 1961) and in viral and cellular membranes and connective tissue (for a more comprehensive review, see Spiro, 1970). Sugar residues commonly found in glycoproteins include D-mannose, D-xylose, D-glucose, L-fucose, N-acetyl neuraminic acid, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine. Carbohydrate is usually attached to the polypeptide backbone by either (a) a Beta-glycosidic bond via the amide of asparagine (Marshall and Neuberger, 1964), or (b) an O-glycosidic bond to serine or threonine (Anderson et al., 1964). Two other forms of linkage, not so far found in viruses, are via (i) hydroxylysine, this linkage is found in collagens of both vertebrates and invertebrates (see Spiro, 1970) and (ii) hydroxyproline, this linkage is found in the cell walls of plants containing upto four residues of L-arabinose (Lamport, 1969).

1.10 Primary structure

The mechanism by which glycoproteins are segregated into their final locations is not yet fully understood. It is believed that these proteins contain all the information that determines their fate (Blobel and Dobberstein, 1975). Blobel (1980) hypothesised that the primary structure of these proteins can be divided into three domains: (i) start transfer signals or signal sequences that initiates transfer of the polypeptide through the lipid bilayer, via the amino-terminus of the nascent polypeptide, (ii) a region that follows this signal sequence through the

membrane that would contain information that determines if the protein should continue to pass through the membrane or how it is folded in the membrane, i.e. an integral transmembrane protein (ITMP), and (iii) a stop transfer region which terminates the transfer of the polypeptide across the membrane. Secreted proteins would presumably have no stop transfer sequence and, therefore, pass through the membrane into the lumen of the endoplasmic reticulum (ER).

1.10.1 Signal sequences

Since the signal hypothesis of Blobel and Dobberstein (1975), signal peptides have been found at the amino-terminus of virtually every nascent glycoprotein, whether it is ultimately secreted or inserted into a cell membrane or viral envelope. The signal peptide is usually cleaved off before completion of synthesis of the polypeptide, a rare exception is ovalbumin which has an internal signal sequence (Lingappa et al., 1979) that is not cleaved off. Kreil (1981) observed certain features common to signal peptides. They are approximately 15-30 residues in length with an internal core of at least nine hydrophobic residues with at least one positive charged residue such as lysine or arganine at the amino-terminal end.

More recently, McGeoch (1985) constructed tests to evaluate candidate signal sequences. These tests successfully predicted 96% of signal sequences present in a pool containing other sequences. It was concluded the following criteria were important for predicting signal sequences: (i) the length (usually 11 residues or less) and net charge of the immediately amino-terminal region which precedes the hydrophobic stretch in the signal sequence, (ii) the length of the uncharged (hydrophobic) region, minimally eight residues in length, (iii) the degree of hydrophobicity of the 8-residue maximal hydrophobic region.

Perlman and Halvorson (1983) have identified a putative signal peptide cleavage region at the carboxy-terminal end of these sequences. Cleavage usually occurs at the fourth, fifth or sixth residue after the end of the hydrophobic core and is identified by the sequence A-X-B, where A is usually alanine, but can be serine, threonine, valine or isoleucine, X is any amino acid and B can be alanine, serine, threonine, glycine or cysteine, cleavage occurs immediately after B.

1.10.2 Anchor sequences

Lingappa et al. (1979) and Chang et al. (1979) predicted the existence of a sequence, distinct from the signal sequence, which stopped the transfer of nascent polypeptides across the cell membrane. Such sequences, which anchor the protein in the cell membrane, are known synonymously as anchor sequences or stop or halt transfer sequences (Blobel, 1980; Boeke and Model, 1982; Sabatini et al., 1982). Sabatini et al. (1982) identified certain features common to anchor sequences. They are usually 20-30 residues in length, are usually, but not necessarily, found near the carboxy-terminus, have a hydrophobic core of approximately twelve residues and are followed by a group of charged residues which would not be expected to enter the lipid bilayer. Perlman and Halvorson (1983) pointed out that anchor sequence hydrophobic cores have less leucine and significantly more glutamic acid and serine residues on a percentage basis than have signal sequence cores. Yost et al. (1983) showed that anchor sequences cannot replace signal sequences. The authors genetically engineered the construction of a fusion protein in which the coding region for an anchor sequence replaced that of a signal sequence of a secreted protein. Transport of the resulting nascent fusion protein through the membrane, instead of being secreted, was predictably stopped by the translocated anchor sequence.

1.10.3 Sites for addition of N- and O-linked oligosaccharides

Eylar (1965) suggested that asparagine (asn) accepted N-linked oligosaccharides where it has a serine (ser) residue next to, or one residue away from it. However, based on the examination of numerous amino acid sequences, Marshall (1974) proposed that asn in the sequence asn-X-ser (thr) was the acceptor (where X is any amino acid). Pless and Lennarz (1977) suggested that this sequence was not sufficient for the addition of N-linked oligosaccharides and showed that some proteins which contain unglycosylated asn-X-ser (thr) sites, could be glycosylated *in vitro* after denaturation. These authors concluded that the addition of carbohydrate to protein requires the exposure of these potential sites.

General rules have not been established to permit predictions as to which ser or thr residues might be used for the addition of O-linked oligosaccharides.

1.11 Synthesis of glycoproteins

Although glycoproteins of enveloped animal viruses are translated from virus specific messenger RNAs, all subsequent processing events are similar to those of host cell glycoproteins (Kornfeld and Kornfeld, 1980). Evidence to suggest that the host cell plays a major role in determining the structure of carbohydrate in viral glycoproteins comes from studies of carbohydrate composition of viruses such as Sindbis (Strauss *et al.*, 1970), vesicular stomatitis (Etchison and Holland, 1974) and influenza (Nakamura and Compans, 1979), grown in different host cell lines. Evidence that HSV glycoproteins use cellular enzymes during oligosaccharide synthesis comes from use of mutant cell lines which lack certain glycosyltransferases (Serafini-Cessi *et al.*, 1983a, b). It was found that the electrophoretic mobility in SDS polyacrylamide gels of the viral glycoproteins synthesised in these cells indicated incomplete glycosylation (see Section 1.20.3).

However, since the E1 protein of Sindbis virus carries both "complex type" and "high mannose type" oligosaccharides (Burke and Keegstra, 1976) (see Section 1.11.2 for explanation of the terms used), the viral glycoprotein itself may influence the type of carbohydrate carried.

As discussed above, the mechanism by which secretory and ITMPs are segregated into their final locations is not fully understood. However, it is known that both appear to be synthesised on membrane bound ribosomes of the rough endoplasmic reticulum (ER) (Morrison and Lodish, 1975; Rothman and Lenard, 1977), both have signal sequences (described in Section 1.10.1) which recognise a signal recognition particle (SRP) consisting of six polypeptides of apparent MW 72K, 68K, 54K, 19K, 14K and 9K daltons (Walter and Blobel, 1980) and one 7S molecule of RNA of about 300 nucleotides (Walter and Blobel, 1982). This SRP has been shown to recognise the signal sequences of nascent secretory proteins (Stoffel et al., 1981) and nascent ITMPs (Anderson et al., 1982). Gilmore et al. (1982a, b) and Meyer et al. (1982) have isolated a "receptor" or "docking protein", a 72K dalton integral membrane protein, which binds the SRP-ribosome complex to form a functional SRP-ribosome-membrane junction complex (Walter and Blobel, 1981) allowing translocation of the nascent polypeptide. An alternative hypothesis proposed by Engelman and Steitz (1981) relies on the thermodynamics of lipid-protein interactions to bring about the spontaneous insertion of proteins into and across the membrane without the participation of specific receptors. The evidence presented by Anderson et al. (1982) and Stoffel et al. (1981) demonstrating that SRPs recognise signal sequences would seem to favour the former theory.

Events after attachment of the SRP-SRP receptor complex to the membrane are poorly understood. However, on the basis that the sequences between the end of the signal peptide and its cleavage site show a high potential for Beta-turns, as calculated from the rules of Chou and

Fasman (1974), it was hypothesised (Steiner et al., 1980; Perlman and Halvorson, 1983) that the nascent polypeptide is inserted into the membrane via a hairpin structure. By this model, the signal sequence is inserted in reverse orientation and is cleaved off on the luminal side of the rough ER by a signal peptidase (Jackson and Blobel, 1977; Walter et al., 1979) at the cleavage site predicted by Perlman and Halvorson (1983). The nascent polypeptide is then translocated through into the luminal side of the rough ER where it can be glycosylated at the appropriate amino acid(s). Glycosylation usually occurs on the nascent polypeptide, as in the case of vesicular stomatitis virus (VSV) (Rothman and Lodish, 1977), but it is not necessarily co-translational (Bergman and Kuehl, 1978; Jamieson, 1977). However, once translocation is under way, secretory proteins ultimately pass through the membrane, while membrane glycoproteins remain embedded by virtue of their anchor sequence. N-linked oligosaccharides may then be modified in the rough and smooth ER (Grinna and Robbins, 1979) and in the Golgi apparatus (Schachter and Roseman, 1980) while being transported to their final destination.

1.11.1 Compartmentalisation of the Golgi apparatus

Studies on processing of the N-linked oligosaccharides have shown that many of the enzymes involved in their processing have a specific location within the Golgi apparatus, an organelle present in all eukaryotic cells. Rothman (1981) first described the Golgi apparatus as having cis and trans compartments. The cis side is nearer the nucleus and adjacent to the rough ER, while the trans side is at the opposite end of a stack of four to six cisternae. More recently, those cisternae lying between the two extreme faces (cis and trans) have been termed the medial cisternae (Griffiths et al., 1983; Bergman and Singer, 1983; Rothman et al., 1984a, 1984b) (fig. 8).

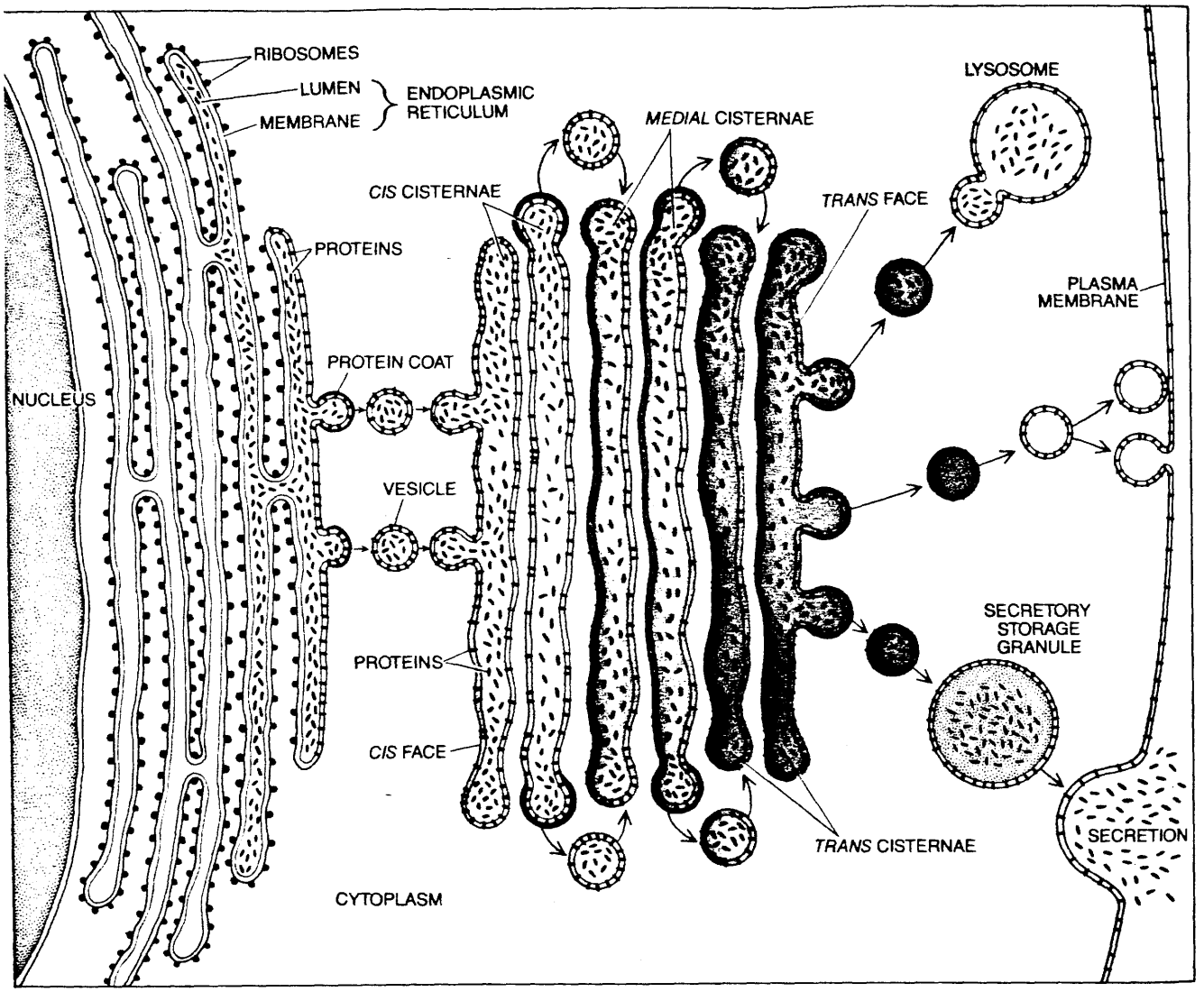

RIBOSOMES
LUMEN
MEMBRANE
ENDOPLASMIC RETICULUM
LYSOSOME
MEDIAL CISTERNAE
CIS CISTERNAE
TRANS FACE
PROTEINS
PLASMA MEMBRANE
PROTEIN COAT
NUCLEUS
VESICLE
SECRETORY STORAGE GRANULE
PROTEINS
CIS FACE
TRANS CISTERNAE
SECRETION
CYTOPLASM

FIGURE 8

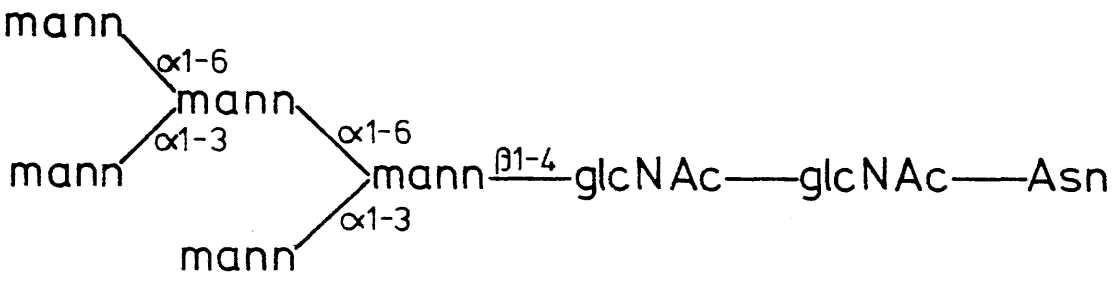
Representation of the Golgi apparatus, the figure is taken from Rothman (1985), showing the cis, medial and trans cisternae. The proteins to be processed in the Golgi apparatus are synthesised on ribosomes that are bound to the endoplasmic reticulum. The completed proteins are either inserted into the ER membrane or pass into its lumen. The proteins are thought to be encapsulated by vesicles which bud from the ER and move toward the Golgi apparatus, fusing with the cis compartment transported by other vesicles to the medial and trans cisternae. Each protein is modified according to its final destination. In the trans compartment the proteins are probably sorted and packaged for delivery, some proteins going to the secretory storage granules, others to lysosomes (large vesicles that contain degradative enzymes) and others to the cellular plasma membrane.

1.11.2 Processing of N-linked oligosaccharides

Oligosaccharide moieties which are N-linked to asparagine can be processed into either high mannose type or complex type oligosaccharides. An example of the smallest typical high mannose type oligosaccharide from human myeloma IgM, (Chapman and Kornfeld, 1979b) is shown in fig. 9a, while fig. 9b shows an example of a typical complex type oligosaccharide from VSV (Reading et al., 1978). It is not known what parameters determine processing of N-linked oligosaccharides. However, it has been suggested that (i) the conformation of two yeast glycoproteins, carboxypeptidase Y and invertase, affects processing of high mannose oligosaccharides (Trimble et al., 1983), (ii) the protein primary structure can determine whether high mannose or complex type oligosaccharides will occur (Baenziger and Kornfeld, 1974; Nakamura and Compans, 1979; Rosner et al., 1980; Sveda et al., 1982), and (iii) a statistical analysis of fifty-six glycoproteins containing either high mannose, complex or both types of oligosaccharide led Pollack and Atkinson (1983) to suggest that N-linked oligosaccharides are more likely to be processed to complex types, the nearer they are to the amino terminus.

It has been established (Waechter and Lennarz, 1976) that the carbohydrate moiety N-linked to asparagine originates from an "en bloc" transfer of an oligosaccharide preformed on the lipid dolichol pyrophosphate (fig. 10). The major precursor oligosaccharide which is assembled on the dolichol pyrophosphate has the composition Glu(3) Mann(9) GlcNAc(2) in cultured Chinese hamster ovary (CHO) cells (Li et al., 1978), NIL-8 hamster fibroblasts (Liu et al., 1979) and in chick embryo cells (Hubbard and Robbins, 1979). Its structure (fig. 11) was almost completely elucidated by Li et al. (1978) except for the linkage of the glucose residues, which were shown to be in the alpha configuration by Liu et al. (1979) and Spiro et al. (1979).

a.


mann
α1-6
mann
α1-3
α1-6
mann
β1-4
glcNAc—glcNAc—Asn
mann
α1-3
mann

b.

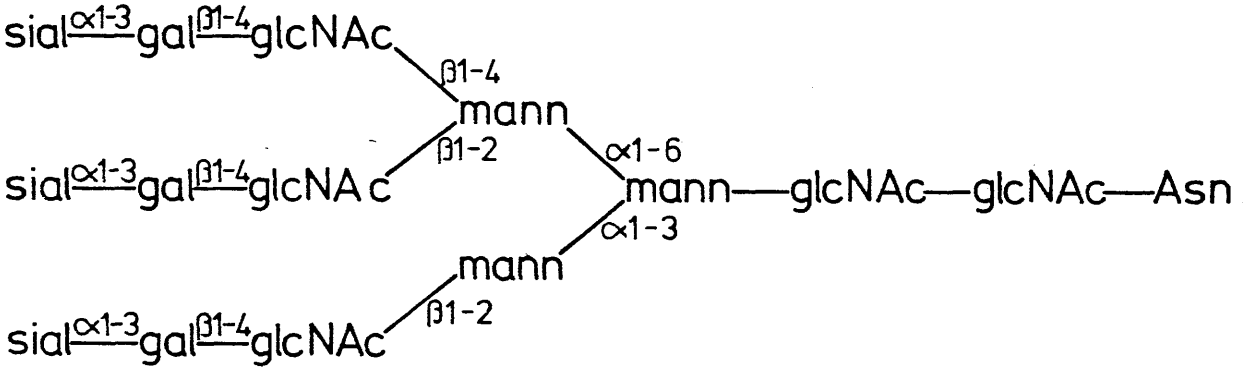
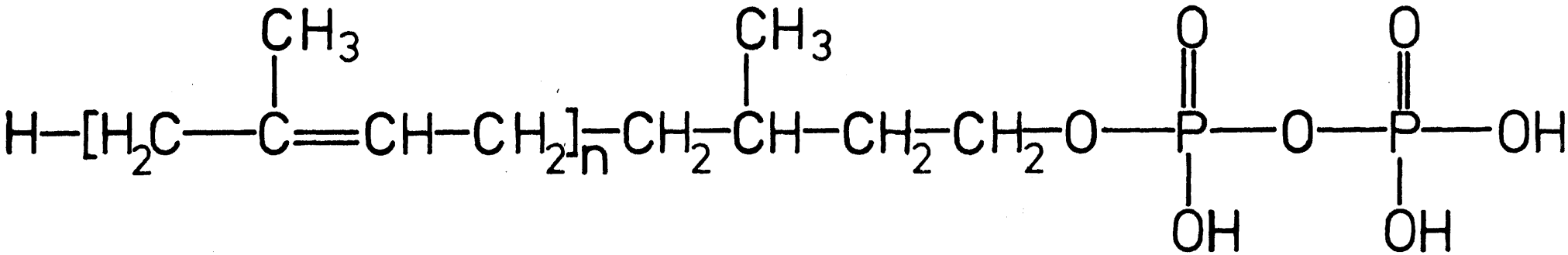

sial α1-3 gal β1-4 glcNAc
β1-4
mann
sial α1-3 gal β1-4 glcNAc
β1-2
α1-6
mann—glcNAc—glcNAc—Asn
α1-3
mann
sial α1-3 gal β1-4 glcNAc
β1-2

FIGURE 9

Glycopeptides showing (a) high-mannose type oligosaccharide from human IgM myeloma protein (Chapman and Kornfeld, 1979b), (b) complex type oligosaccharide of VSV G protein (Reading et al., 1978). Abbreviations used are:

Mannose (mann), N-acetylglucosamine (glcNAc), asparagine (Asn), sialic acid (sial), galactose (gal).



dolichol pyrophosphate. In mammals, n=18–20

FIGURE 10

Chemical formula of dolichol pyrophosphate

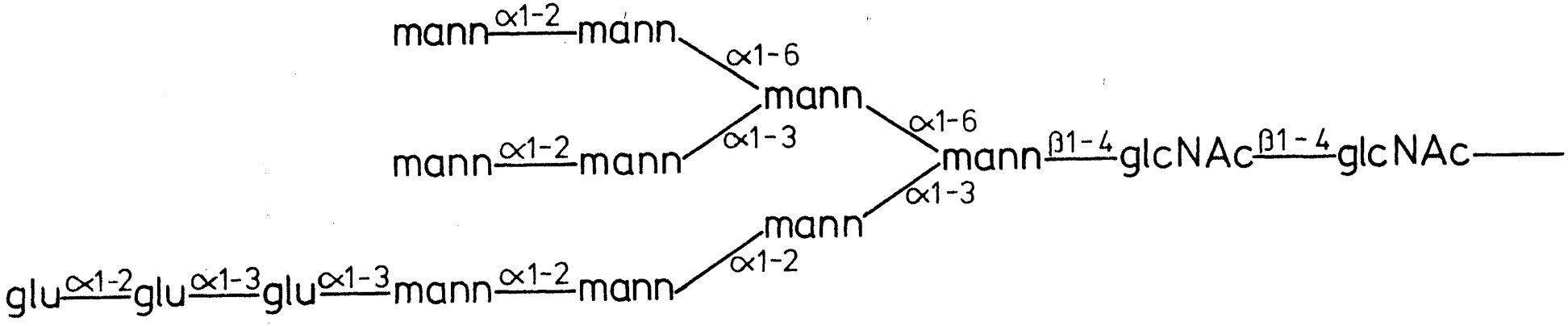

mann α1-2 mann
α1-6
mann
α1-6
mann α1-2 mann
α1-3
mann β1-4 glcNAc β1-4 glcNAc
α1-3
mann
α1-2
glu α1-2 glu α1-3 glu α1-3 mann α1-2 mann

FIGURE 11

The major precursor oligosaccharide elucidated by Li et al. (1978) and Liu et al. (1979) that is preformed on dolichol pyrophosphate before being transferred to asparagine.

Abbreviations used are:

Glucose (glu), mannose (mann), N-acetylglucosamine (glcNAc)

Processing can be divided into four stages: (1) enzymatic removal of the three glucose residues, (2) enzymatic removal of the four alpha 1-2 bonded mannose residues to yield the smallest high mannose structure (fig. 9a), (3) enzymatic removal of another two mannose residues to yield the core structure of complex oligosaccharides, and (4) synthesis of complex oligosaccharides. Each of these stages is discussed below in more detail:

Step 1

Processing is initiated by the rapid enzymatic removal of the three glucose residues. Hubbard and Robbins (1979) presented evidence that this can occur within 5min. in chick embryo fibroblast cells. Grinna and Robbins (1979) isolated two glycosidase activities from the rough and smooth ER of microsomal membranes of rat liver. One of the enzymes catalysed the removal of only the terminal residue, the second enzyme catalysed the sequential removal of the remaining two glucose residues, thus leaving the oligosaccharide of composition mann(9) glcNAc(2). A glycopeptide of thyroglobulin retains this structure (Ito et al., 1977), but usually it is processed further into either smaller high mannose type or complex type oligosaccharides, both of which exhibit heterogeneity (Kornfeld and Kornfeld, 1976).

Step 2

Tulsiani et al. (1977) and Opheim and Touster (1978) presented evidence that a mannosidase activity isolated from rat liver Golgi membranes was involved in the metabolism of glycoprotein oligosaccharides. Tabas and Kornfeld (1979) and Tulsiani et al. (1982a) showed that there were at least two mannosidase activities (1A and 1B) in rat liver Golgi membranes which hydrolyse the alpha 1-2 bonded mannose residues.

Kornfeld et al. (1978) presented evidence that the four alpha 1-2 bonded mannose residues are removed in a specific order. This order may be different in specific glycoproteins (Chapman and Kornfeld, 1979a; Chapman and Kornfeld, 1979b; Hubbard and Ivatt, 1981).

Step 3

Synthesis of complex oligosaccharides is initiated by conversion of the high mannose type oligosaccharide to a core oligosaccharide, glcNAc-mann(3)-glcNAc(2) (Kornfeld and Kornfeld, 1980). Two enzymes are involved in this step. First, N-acetylglucosamine transferase I (Tabas and Kornfeld, 1978; Harpaz and Schachter, 1980a) catalyses the transfer of a single residue of glcNAc to the terminal residue of the high mannose oligosaccharide mann(5)-glcNAc(2) - at the position shown in fig. 12a. Dunphy and Rothman (1983) have presented evidence showing compartmentalisation of the N-linked oligosaccharide processing in the Golgi apparatus and localised this enzyme to the cis side of the Golgi stack in CHO cells. Only when the oligosaccharide of structure glcNAc(1) mann(5) glcNAc(2) (fig. 12a) has been formed will the second enzyme, mannosidase II, cleave off the other two terminal mannose residues (Tabas and Kornfeld, 1978; Harpaz and Schachter, 1980b; Tulsiani et al., 1982a) (fig. 12b). Mannosidase II is also located in the cis side of the Golgi apparatus (Dunphy and Rothman, 1983).

Step 4

The final stage is the elongation to form the complex oligosaccharides by the sequential action of specific glycosyltransferases. These are: (a) N-acetylglucosaminyltransferases, (b) galactosyl-transferases, (c) fucosyltransferases, and (d) sialyltransferases. The one enzyme-one linkage concept (Hagopian and Eylar, 1968) would dictate that

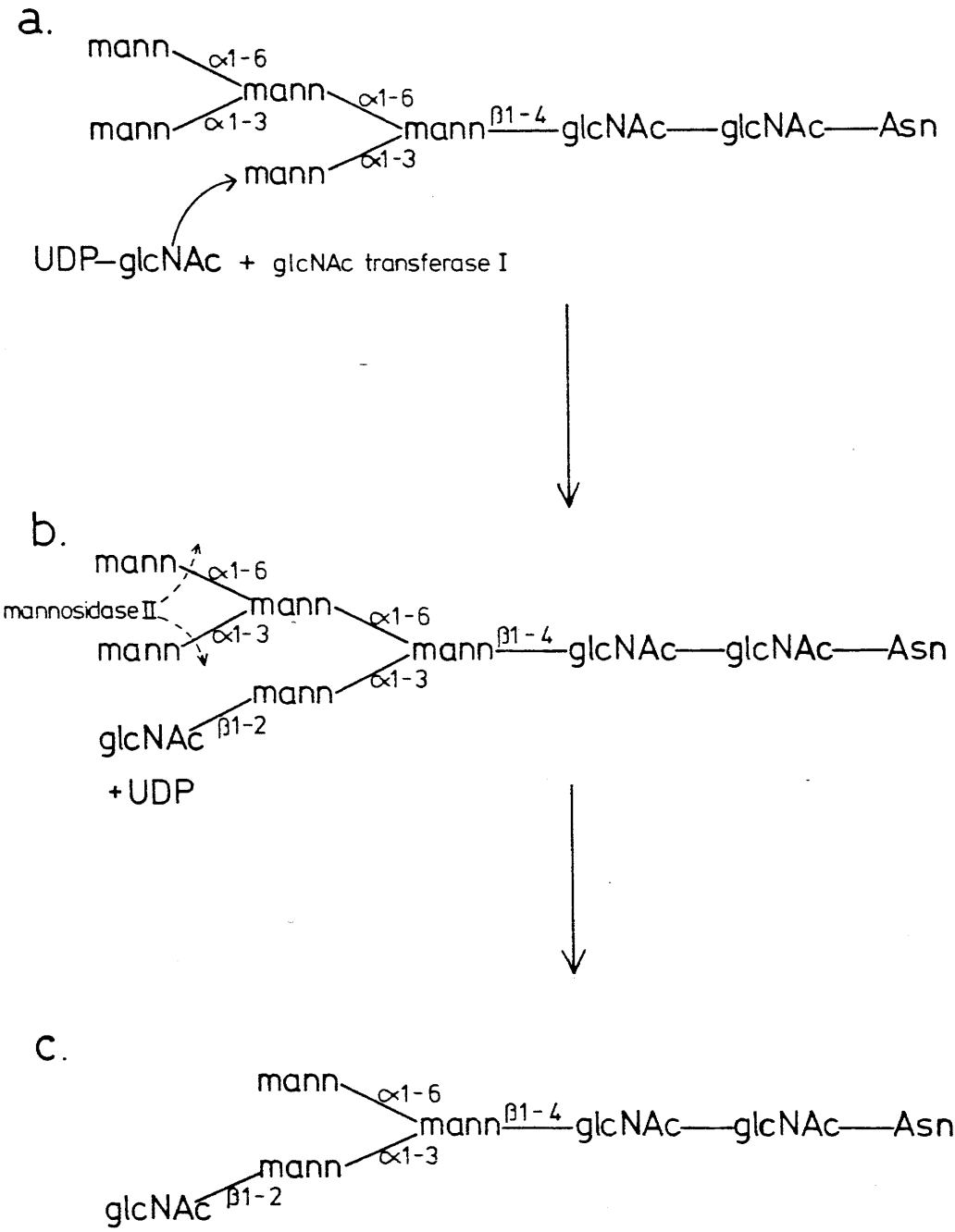

a.
mann
α1-6
mann
α1-6
mann
α1-3
mann
β1-4
glcNAc—glcNAc—Asn
α1-3
mann
UDP–glcNAc + glcNAc transferase I
b.
mann
α1-6
mannosidase II
mann
α1-6
mann
α1-3
mann
β1-4
glcNAc—glcNAc—Asn
α1-3
mann
glcNAc
β1-2
+UDP
c.
mann
α1-6
mann
β1-4
glcNAc—glcNAc—Asn
mann
α1-3
glcNAc
β1-2

FIGURE 12

Processing of N-linked oligosaccharides from the smallest high-mannose type to the core for the complex type oligosaccharide.

(a) the high-mannose oligosaccharide which is the substrate for the enzyme N-acetylglucosaminyl transferase I. The donor is UDP-glc NAc;

(b) the product of the reaction described in (a) which is the target (indicated by dotted arrows) for mannosidase II;

(c) the product of the reaction described in (b) which is the core for complex type oligosaccharides.

Abbreviations used are as for figs. 11 and 13.

one specific transferase is required for each specific glycosidic linkage found in nature; that is, multiple forms of a particular sugar transferase exist. Goldberg and Kornfeld (1983) presented evidence that the order in which the transferases are distributed from the cis to the trans Golgi membranes is identical with the sequence that the enzymes appear to act in vivo.

Beyer et al. (1981) suggest that at least five distinct N-acetylglucosaminyl (glcNAc) transferases may be involved in the elongation of complex oligosaccharides. To date, three have been identified:

(i) glcNAc transferase I (Tabas and Kornfeld, 1978; Harpaz and Schachter, 1980a) (described in step 3);

(ii) glcNAc transferase II which has been partially purified by Harpaz and Schachter (1980a). These authors proposed the reaction in fig. 13A where the acceptor is the core of complex oligosaccharides. The glcNAc residue is added onto the remaining terminal mannose residue resulting in an oligosaccharide having two branches (biantennary);

(iii) glcNAc transferase IV was identified by Cummings et al. (1982), who proposed the reaction in fig. 13B. GlcNAc is added via a beta-1-6 linkage to form a three (tri) or four branched (tetraantennary) oligosaccharide. The acceptor oligosaccharide must have both mannose residues substituted with glcNAc.

Based on known oligosaccharide structures (Kornfeld and Kornfeld, 1976; Paulson et al., 1978), three fucosyltransferases are required:

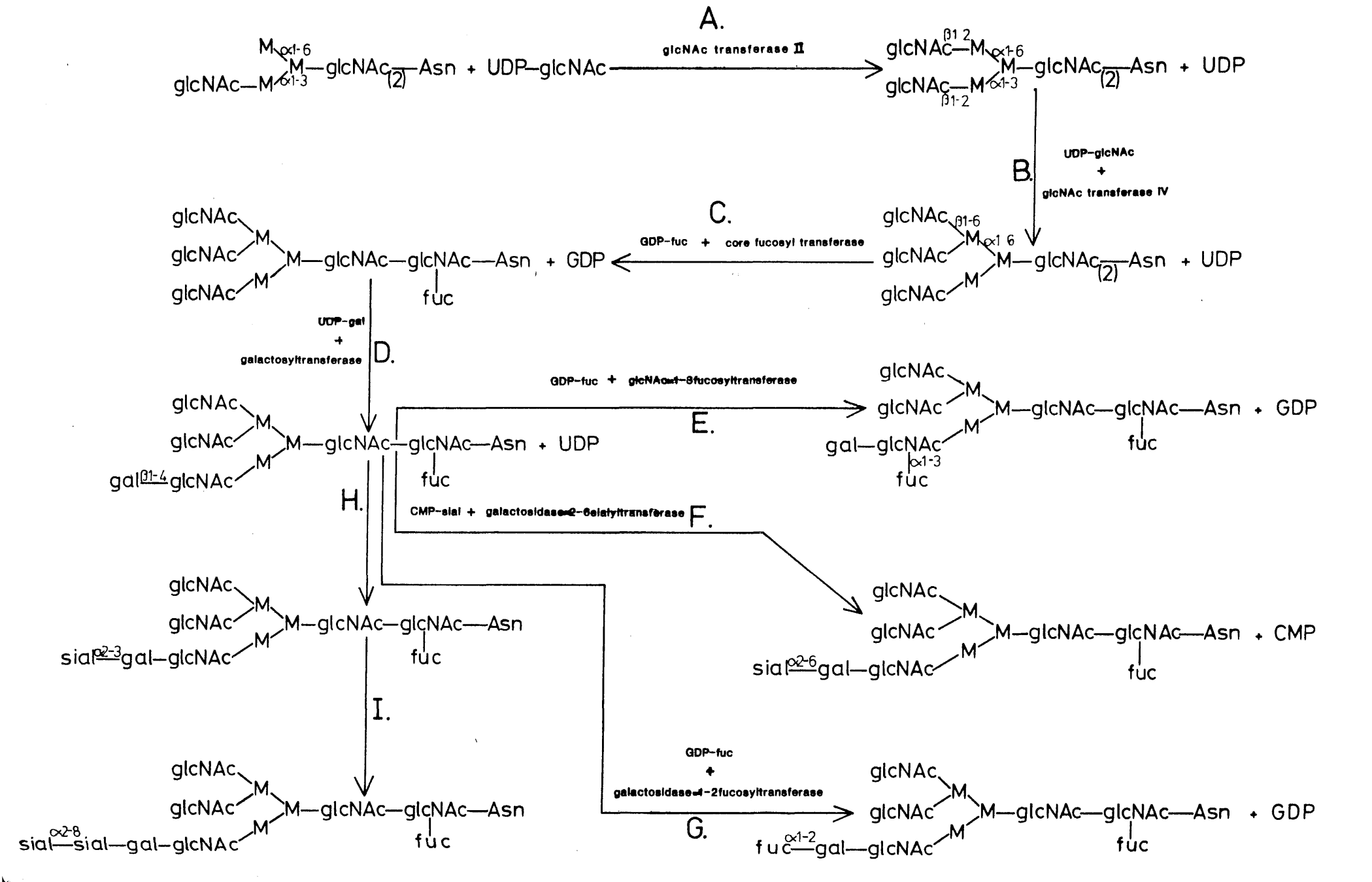

A.
glcNAc transferase II
UDP-glcNAc
+
glcNAc transferase IV
B.
C.
GDP-fuc + core fucosyl transferase
UDP-gal
+
galactosyltransferase
D.
GDP-fuc + glcNAcα1-3fucosyltransferase
E.
CMP-sial + galactosidaseα2-6sialyltransferase
F.
GDP-fuc
+
galactosidaseα1-2fucosyltransferase
G.
H.
I.
Asn
UDP
GDP
CMP
fuc
gal
glcNAc
sial

FIGURE 13

Putative pathway for the synthesis of a complex oligosaccharide N-linked to asparagine. The reactions shown are not necessarily the only acceptor and product for each enzyme (see references in text, Section 1.11.2).

Abbreviations used are as for figs. 9, 11 and 12 except M = mannose and fuc = fucose.

(i) Beta-N-acetylglucosamine-1-N-asparagine-fucosyltransferase (core fucosyltransferase) catalyses the reaction in fig. 13C (Wilson et al., 1976). It has the specificity to act after glcNAc transferase I but before galactosyltransferase (Wilson et al., 1976; Beyer et al., 1981);

(ii) N-acetylglucosaminide-alpha-1-3-fucosyltransferase catalyses the reaction in fig. 13E (Shen et al., 1967; Prieels et al., 1977; Paulson et al., 1978);

(iii) Beta-galactoside-alpha-1-2-fucosyltransferase catalyses the reaction in fig. 13G (Shen et al., 1967; Bosmann et al., 1968; Schenkel-Brunner et al., 1975).

Galactosyltransferase (Fleischer et al., 1969) catalyses the addition of a galactose (Gal) residue to glcNAc via a Beta-1-4 linkage (fig. 13D) (Rao and Mendicino, 1978).

At least three different sialyltransferases are required for the known sialic acid linkages (Kornfeld and Kornfeld, 1976; Finne et al., 1977):

(i) Beta-galactoside-alpha-2-6-sialyltransferase catalyses the reaction in fig. 13F (Paulson et al., 1977) and is the only sialyltransferase so far identified which is specific for N-linked oligosaccharides. Paulson et al. (1978) presented evidence to show that this enzyme purified from bovine colostrum and N-acetylglucosaminide-alpha-1-3-fucosyltransferase, purified from human milk (Prieels et al., 1977) use the same substrate, i.e. gal-beta-1-4-glcNAc (the implications of this observation are not known).

(ii) The linkage sial-alpha-2-3-gal has been identified in N-linked oligosaccharides by Carlson et al. (1973) and Thomas and Winzler

(1971).

(iii) The linkage sial-alpha-2-8-sial has been identified by Finne et al. (1977) and Spiro (1973). The enzymes which catalyse the formation of the latter two linkages have not been identified (see figs. 13H and 13I for putative structures).

1.11.3 Biosynthesis of oligosaccharides O-linked to serine or threonine

Unlike N-linked oligosaccharides which are added "en bloc" from a lipid carrier, the oligosaccharides O-linked to serine or threonine are synthesised one at a time from nucleoside sugars, directly onto the protein (Schachter, 1979; Beyer and Hill, 1982). The constituent sugars are D-galactose (gal), N-acetyl-D-galactosamine (galNAc), N-acetyl-D-glucosamine (gluNAc), L-fucose, sialic acid and D-xylose, but very rarely D-mannose (Pierce and Parsons, 1981). The initial sugar residue added to serine or threonine is usually galNAc (McGuire and Roseman, 1967) and most O-linked oligosaccharides contain a core of gal-beta-1-3-galNAc - ser/thr (Kornfeld and Kornfeld, 1980).

Several groups have investigated the site of addition of the initial sugar residue. The enzyme galNAc transferase that catalysed the addition of the initial gal NAc residue has been found in a fraction consisting of smooth ER and Golgi membranes, with relatively little activity in the rough ER membrane fraction (Ko and Raghupathy, 1972; Hanover et al., 1980) indicating that synthesis of O-linked oligosaccharides does not commence until after the polypeptide synthesis is complete. Although Strous (1979) reported O-glycosylation of epithelial cell glycoproteins occurs at the level of nascent chains and it is possible that the site of glycosylation differs in various biological systems, this author did not directly establish that the galNAc detected in preparations of nascent chains, was not derived from contaminating mature O-linked glycoproteins.

The glycosyltransferases that catalyse the addition of the terminal sugar residues to O-linked oligosaccharides are not as well characterised as those that add terminal sugars to N-linked oligosaccharides, but probably reside in the Golgi apparatus (Schachter, 1979).

No rules predicting which of the serines and threonines will be O-glycosylated have been formulated.

1.11.4 Sulphation of proteins

Sulphate is incorporated into the proteins of many viruses, such as the HN and F glycoproteins of simian virus 5 and sendai virus (paramyxoviruses), the glycoprotein G of VSV (rhabdovirus), the E1 and E2 glycoproteins of sindbis virus (togovirus) and glycoproteins of apparent MW 52000 and 69000 of rauscher leukaemia virus (oncornavirus) (Pinter and Compans, 1975), the HA glycoprotein of influenza virus (orthomyxovirus) (Compans and Pinter, 1975), a glycoprotein of apparent MW 99000 of pseudorabies (herpesvirus) (Erickson and Kaplan, 1973; Pennington and McCrae, 1977) and a glycoprotein of apparent MW 44000 of myxovirus (leporipoxvirus) (Pennington et al., 1982).

The site of attachment (oligosaccharide moiety or polypeptide backbone) of sulphate to each of the glycoproteins mentioned above, is not known, nor is the role that the attached sulphate plays during the maturation of viruses.

It has been shown that sulphate can covalently link to the polypeptide backbone and/or the sugars of the oligosaccharide moiety. So far, tyrosine is the only amino acid to which attachment of sulphate has been demonstrated (Huttner, 1982, 1984). The sulphate is attached as an O-sulphate ester link (Huttner, 1984). The ten tyrosine-sulphated proteins studied to date (Huttner, 1982, 1984; Rosa et al., 1985) have all been shown to be secreted proteins. Lee and Huttner (1983) identified a

tyrosylprotein sulfotransferase which catalyses the addition of sulphate onto tyrosine of four proteins in pheochromocytoma cells. This enzyme has been isolated from the Golgi apparatus (Lee and Huttner, 1984).

The sulphate covalently linked to the oligosaccharides was demonstrated to be attached to (i) the six position of glcNAc adjacent to asparagine in the HN glycoprotein of simian virus 5 (Prehm et al., 1979); (ii) into the four position of galactose in the N-linked oligosaccharides of ovine luteinizing hormone (Bedi et al., 1982); (iii) the six position of galactose in both N- and O-linked oligosaccharides of the hormone human chorionic gonadotropin (Pierce and Parsons, 1981); or (iv) into galNAc in N-linked oligosaccharides of the pituitary hormone, bovine lutropin (Parsons and Pierce, 1980).

Farquar and Palade (1981) suggested that oligosaccharide-sulphation plays a role in the packaging of secretory proteins, and it has been shown (Karp and Solursch, 1974) that cultivation of sea urchin embryos is arrested at the blastula stage if they are grown in the absence of sulphate.

1.11.5 Acylation

Schmidt et al. (1979) have described the addition of ^{3}H-palmitate to the E1 and E2 glycoproteins of Sindbis virus. E1 contains two moles of fatty acid per mole of protein and E2 contains five or six moles of fatty acid per mole of protein. It has been suggested (Schmidt et al., 1979; Schmidt and Schlesinger, 1980) that the addition of fatty acid, which is covalently linked to the polypeptide backbone, takes place in the Golgi complex.

Schmidt and Schlesinger (1979) presented evidence which showed that ^{3}H-palmitate is incorporated into the VSV G protein. This linkage is probably a thioester bond to a cysteine residue (Rose et al., 1984). This post-translational modification is not exclusive to glycoproteins, but is

found in non-glycosylated membrane proteins, such as SV40 T antigen (Henning and Lange-Mutschler, 1983) and the transferrin receptor in cultured human cells (Omary and Trowbridge, 1981).

A variation of this post-translational modification is seen in the transforming protein, $p60^{src}$ of Rous Sarcoma Virus. Myristic acid is attached to the polypeptide backbone but via an amide bond (Buss and Sefton, 1985), unlike the ester bond which attaches palmitic acid.

1.12 Drugs used for investigating post-translational modifications functions and activities of glycoproteins

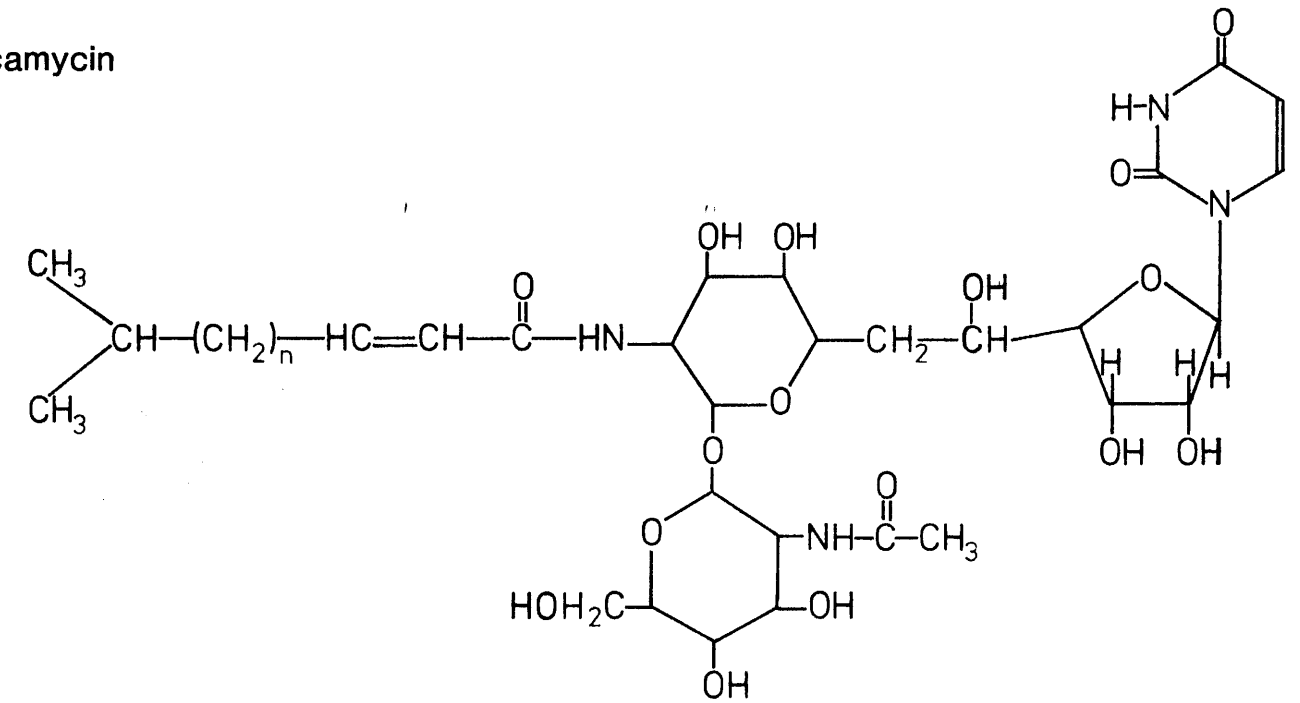
Tunicamycin

The structure of the antibiotic tunicamycin (TM) is shown in fig. 14 (Takatsuki et al., 1977; Ito et al., 1980). It prevents formation of dolichol pyrophosphate-N-acetylglucosamine (Tkacz and Lampen, 1975), the high mannose oligosaccharide by inhibiting the transfer of glcNAc from UDP-glcNAc to dolichol phosphate. However, O-glycosylation still proceeds in TM-treated cells (Gahmberg et al., 1980; Butters and Hughes, 1980; Speake and White, 1979). The release of infectious virus is reduced in the presence of TM. This has been observed for VSV and Sindbis (Leavitt et al., 1977), SFV and fowl plague virus (FPV) (Schwarz et al., 1976), Snowshoe Hare Bunyavirus (Cash et al., 1980), Newcastle Disease Virus (NDV) (Morrison and Simpson, 1980), measles virus (Stallcup and Fields, 1981), HSV (Pizer et al., 1980) and rous sarcoma virus (Stohrer and Hunter, 1979).

2-deoxy-D-glucose

Kilbourne (1959) demonstrated that 2-deoxy-D-glucose (fig. 14) interferes with the multiplication of influenza virus, however, it was subsequently shown that viral polypeptide synthesis is not affected, but glycoprotein production is affected (Kaluza et al., 1972; Ghandi et al.,

(a.) Tunicamycin



(b.) 2-deoxy-D-glucose

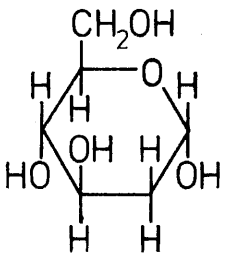


FIGURE 14

Chemical formulae for

(a) tunicamycin, a nucleoside antibiotic. It is a mixture of homologous antibiotics. The homologues differ in their fatty acid component. The most abundant homologues are when n = 9, 10, 11 and 12;

(b) 2-deoxy-D-glucose, an analogue of mannose.

1972; Klenk et al., 1972) suggesting that it is the glycosylation step that is inhibited. This was confirmed by Kaluza et al. (1973); Knowles and Person (1976) and Courtney et al. (1976) demonstrated that 2-deoxy-D-glucose acted as an analogue of D-mannose. The authors proposed that this sugar analogue prevents elongation of the oligosaccharide chain. Comparison of evidence presented by Glorioso et al. (1983) who used 2-deoxy-D-glucose and by Norrild and Pederson (1982) who used tunicamycin shows that use of either drug results in underglycosylated HSV-1 glycoproteins, gC and gD, present on the surface of infected cell membrane, but not gB. Furthermore, the MWs of the underglycosylated precursors, resulting from use of either drug, are similar, suggesting that 2-deoxy-D-glucose and tunicamycin have similar effects on glycosylation.

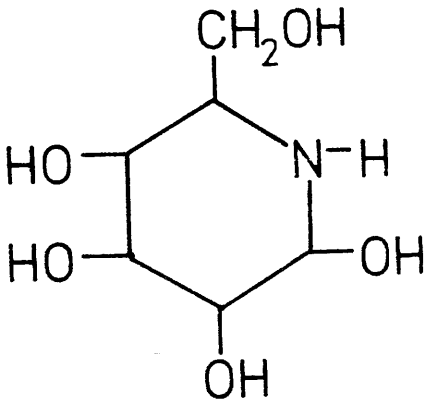
After transfer of the oligosaccharide to asparagine, the oligosaccharides are usually processed to the complex-type. With the drugs available, this processing can be inhibited at three stages:

First, Nojirimycin (fig. 15a) (Niwa et al., 1970) and 1-deoxynojirimycin (Saunier et al., 1982) inhibit glycosidase I, and also inhibits the formation of the dolichol-linked oligosaccharide (Romero et al., 1983; Gross et al., 1983). However, N-methyl-1-deoxynojirimycin inhibits only glucosidase I (Romero et al., 1983).

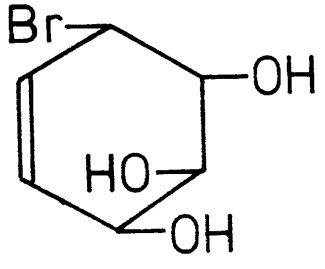
Datema et al. (1982), who studied glycosylation in infected chick embryo cells with FPV, showed that the second stage of processing, release of the innermost glucose residue on the high mannose transferred oligosaccharide (glc(3) mann(9) glcNAc(2)), can be inhibited with bromoconduritol (fig. 15b) (Legler et al., 1977) and suggested that the drug interferes with the active site of glucosidase II. The authors also showed that the action of bromoconduritol does not interfere with the enzymic activity of mannosidase I on the remaining two terminal mannose residues.

The third stage at which N-linked oligosaccharides processing can

(a.) Nojirimycin.



(b.) Bromoconduritol.



(c.) Swainsonine

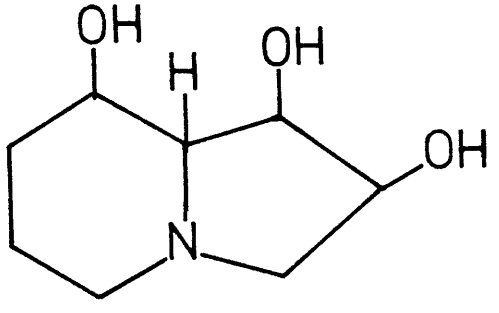


FIGURE 15

Chemical formulae of inhibitors of processing of the N-linked oligosaccharides. The mode of action of each inhibitor is described in Section 1.12.

be inhibited is during removal of the mannose residues. Using the drug swainsonine (fig. 15c), isolated from the plant "swainsona canescens" by Colegate et al. (1979). Elbein et al. (1981) first showed that it prevents the formation of complex oligosaccharides in CHO and Madin-Darby Canine Kidney (MDCK) cells. More recently, Tulsiani et al. (1982b) presented evidence to show that swainsonine specifically inhibits mannosidase II, purified from rat liver Golgi membranes, which catalyses the reaction described in Section 1.11.2, step 3.

Monensin has been used to study the transport of the VSV G glycoprotein and the E1 and E2 glycoproteins of Sindbis virus (Johnson and Schlesinger, 1980) and also the E1 and E2 glycoproteins of SFV (Griffiths et al., 1983; Quinn et al., 1983), a virus closely related to Sindbis. Griffiths et al. (1983) suggested that monensin does not inhibit any specific reaction in oligosaccharide synthesis, but rather it blocks the transport of membrane bound proteins at a number of stages. However, the mode of action of monensin is far from clear since the effect of the drug depends on the cell type used. Thus, in SFV-infected Baby Hamster Kidney (BHK) cells grown in the presence of monensin, transport from the medial to the trans Golgi is inhibited (Griffiths et al., 1983; Quinn et al., 1983), however, when SFV-infected chick embryo cells are grown in the presence of monensin (Pesonen and Kaariainen, 1982), transport of membrane bound proteins is inhibited after they have left the Golgi cisternae. Whether these differences reflect different efficiencies of transport of the drug in the two cell types or whether different targets exist in different cell types is not known.

1.13 Glycosidic enzymes as tools for structural studies on oligosaccharides

Glycosidic enzymes fall into two categories: exoglycosidases, and

endoglycosidases.

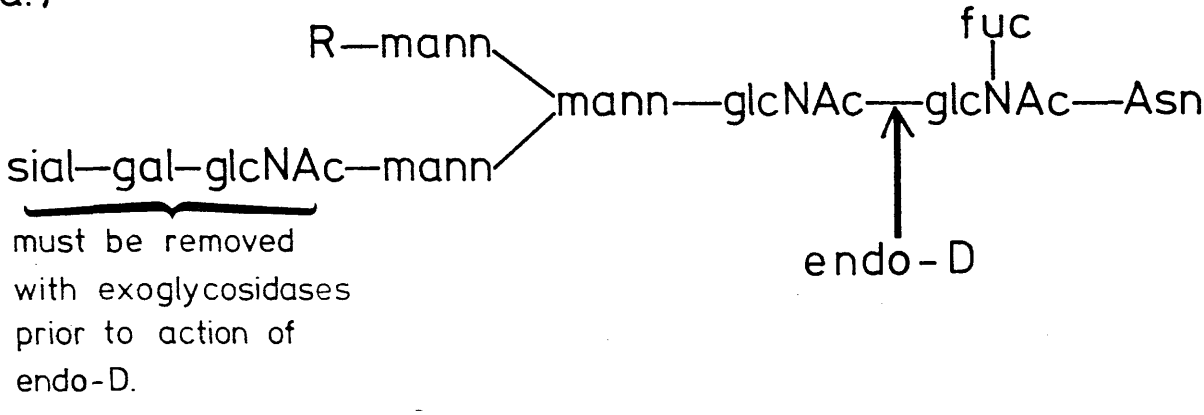
Exoglycosidases cleave only terminal sugar residues and can reveal the configuration of the oligosaccharide by the specificity of the glycosidase for alpha and beta linkage. The specificity of the exoglycosidases, glucosidases I and II and mannosidases IA, IB and II have been reported in Section 1.11.2. Others include neuraminadase (Cassidy et al., 1965) which cleaves terminal sialic acid, beta-D-galactosidase (Li and Li, 1968; Spiro, 1962) which cleaves terminal beta-linked galactose and alpha-L-fucosidase (Bahl, 1970) which cleaves terminal alpha-linked fucose.

Endoglycosidases cleave within the oligosaccharide chain. The two most frequently used to elucidate oligosaccharide structure are endo-beta-N-acetylglucosaminidase-D (endo-D) (Muramatsu, 1971) and endo-beta-N-acetylglucosaminidase-H (endo-H) (Tarentino et al., 1972; Tarentino and Maley, 1974). Their cleavage sites are shown in fig. 16. Endo-D is suitable for studies on glycoproteins with complex oligosaccharides (Koide and Muramatsu, 1974) in which sialic acid, galactose and N-acetyl-glucosamine have been removed with exoglycosidases from the mannose alpha-1-3-linked to the innermost mannose residue (Tai et al., 1975). Endo-H is suitable for studies on glycoproteins with high-mannose type oligosaccharides (Tarentino et al., 1972; Tarentino and Maley, 1974). Another useful endoglycosidase is alpha-D-N-acetylgalactosaminyl-oligosaccharidase (NAGO) (Huang and Aminoff, 1972) which cleaves desialylated oligosaccharide chains that are linked O-glycosidically through alpha-D-N-acetylgalactosaminyl residues to serine or threonine residues of the protein (fig. 16).

1.14 Lectins

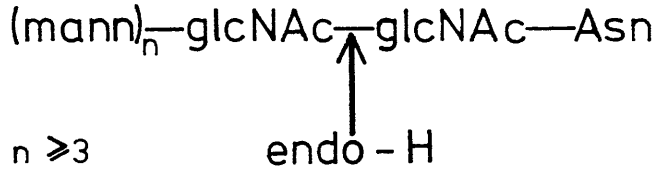
Lectins are proteins which are characterised by their property to

(a.)



must be removed with exoglycosidases prior to action of endo-D.

(b.)



$n \geqslant 3$

(c.)

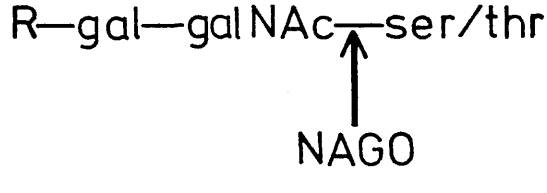


FIGURE 16

Mode of enzymic action of (a) endo-D, (b) endo-H and (c) N-acetylgalactosamine oligosaccharidase (NAGO). (a) Endo-D cleaves complex oligosaccharides (Koide and Muramatsu, 1974) in which the mannose residues alpha- 1-3 linked to the innermost mannose residue is unsubstituted (Tai et al., 1975). R can contain sial, gal and glcNAc. (b) Endo-H can cleave high-mannose type oligosaccharides (Tarentino et al., 1972; Tarentino and Maley, 1974). (c) NAGO cleaves O-linked oligosaccharides at the site shown (Huang and Aminoff, 1972).

TABLE 3

Binding characteristics of some lectins

lectin	source	sugar specificity	Ref[a]
concanavalin A	jack bean	glu; mann	1, 2
soybean agglutinin	soybean	gal; galNAc	1, 2
helix pomatia lectin	vineyard snail	galNAc	3
wheat germ agglutinin	wheat germ	glcNAc	1, 2
hepatic lectin	mammalian liver	gal; galNAc	4

a Key to references (REF)

1 Sharon and Lis, 1972

2 Goldstein and Hayes, 1978

3 Axelsson et al., 1978

4 Achord et al., 1978

Abbreviations used are:

glucose (glu), mannose (mann), galactose (gal), N-acetylgalactosamine (galNAc), N-acetylglucosamine (glcNAc)

bind the sugar residues of glycoproteins and are found in all categories of living organisms. Table 3 lists several lectins and their sugar specificities.

Hamblin and Kent (1973) first showed that lectins are responsible for the binding of nitrogen fixing bacteria to the root hairs of plants via a lectin-bacteria interaction. Since then, many lectins have been found to be involved in this type of interaction, each lectin binding a specific strain of nitrogen fixing bacteria (for review, see Sharon and Lis, 1982).

A hepatic lectin has been isolated from rat and rabbit liver (Stockert et al., 1974; LaBadie et al., 1975; Pricer and Ashwell, 1976) that binds desialylated serum glycoproteins that terminate in galactose or N-acetylgalactosamine residues. The desialylated glycoproteins are then transferred from the circulation to the hepatocellular lysosomes, where catabolism occurs (Hubbard et al., 1979).

However, the endogenous function of most lectins is not known. They were initially used in serological blood typing because different lectins preferentially agglutinate red blood cells of different types (Watkins and Morgan, 1952; Watkins, 1972). Some plant lectins can induce mitogenesis (Nowell, 1960), consequently these proteins became widely used reagents in immunology. Lectins have also been used extensively as affinity reagents for purifying glycoproteins.

1.15 Functions of the carbohydrate moiety

The approaches most frequently used to investigate the function of the oligosaccharides of glycoproteins are, elimination of the sugar moiety by glycosidases (see Section 1.13), prevention of attachment of the sugar moiety to the polypeptide (see Section 1.12), the use of inhibitors of processing to modify the oligosaccharide structure on the polypeptide (see Section 1.12), and isolation of cell mutants with defects in the synthesis of glycosyltransferases (Meager et al., 1975, 1976; Gottlieb et al., 1979).

Using these approaches, the following roles have been proposed for the oligosaccharide moiety of glycoproteins: (i) Maintenance of protein conformation and solubility, (ii) Stabilisation of the polypeptide against proteolysis, (iii) Mediation of biological activity, and (iv) Intracellular sorting and externalisation.

The oligosaccharide moiety can represent a significant alteration in the size and structure of the polypeptide and may modify such physicochemical properties as conformation and solubility. Gibson et al. (1978, 1979) observed that differences in the properties of non-glycosylated G protein of VSV, synthesised in infected cells in the presence of TM, depended on the temperature at which it was synthesised. Non-glycosylated G protein extracted from cells infected at 38°C was insoluble in a buffer containing Triton-X-100, whereas non-glycosylated G protein synthesised at 30°C remained in solution at the top of a sucrose gradient (as did glycosylated G protein). Furthermore, when the non-glycosylated G protein, synthesised at either 30°C or 38°C was extracted with 6M guanidine hydrochloride (GuHCl), containing Triton-X-100, and then dialysed to remove the GuHCl, the G protein dialysed at +4°C remained in solution, while those molecules dialysed at 38°C did not, demonstrating that non-glycosylated G protein undergoes a conformational change at elevated temperatures resulting in aggregation and insolubility.

Evidence suggesting that processing of the oligosaccharide chains confers conformational change on glycoproteins was presented by Schwarz (1979) who raised antibodies against non-glycosylated envelope proteins of SFV. The antibodies did not react with the native glycosylated counterparts of SFV, but did react with the high mannose precursor glycopeptide of the envelope protein. This suggests that the formation of complex oligosaccharides confers conformational features on the mature glycoprotein which are not present on the non-glycosylated species.

The way in which the oligosaccharide moiety protects against proteolysis is not known. Studies on the sensitivity of RNase A and RNase B to proteolysis by trypsin (reviewed by Olden et al., 1985) suggest that proteolysis can be modulated by the physical accessibility to the cleavage site of the protein, since RNase A and RNase B are similar except for the presence of a single high mannose type oligosaccharide on asparagine 34 of RNase B (Plummer and Hirs, 1964). RNase B was 6-10 times more resistant to trypsin digestion than non-glycosylated RNase. Furthermore, RNase B with smaller oligosaccharide chains were sensitive to proteolysis at rates proportional to the size of the oligosaccharide, indicating that the proximity of the carbohydrate to the cleavage site is important.

Another example in which proteolysis is controlled by the presence of oligosaccharide chains is the cleavage of the HA glycoprotein of influenza virus into subunits HA1 and HA2 (Klenk et al., 1975; Lazarowitz and Choppin, 1975). Several investigators (Schwarz et al., 1976; Nakamura and Compans, 1978) have observed that the non-glycosylated HA glycoprotein is highly susceptible to proteolytic degradation.

The presence of the oligosaccharide moiety has been shown to be essential for the biological activity of a few glycoproteins. For example, carbohydrate-deficient monoclonal hapten-specific antibodies behaved like normal antibodies with respect to antigen- and protein A-binding properties, but lost their ability to activate complement, to bind to Fc-receptors and to induce cytotoxicity (Nose and Wigzell, 1983) indicating that the loss of oligosaccharides from immunoglobulins have a profound effect on their biological activity.

Transport of glycoproteins to specific organelles may require the presence of information inherent in the glycoprotein. Leavitt et al. (1977) showed that non-glycosylated G protein of VSV, synthesised in infected cells in the presence of TM, is not transported to the cell surface,

suggesting that the oligosaccharide moieties of glycoprotein G are required for transport to the cell surface.

Although the secretion of glycoproteins depends on the absence of a membrane anchoring sequence (see Section 1.10.2), usually located near the carboxy-terminus of the polypeptide, oligosaccharide processing may be important for the secretion of, at least, some glycoproteins. Secretion of the alpha-1-proteinase inhibitor was inhibited by concentrations of 1-deoxynojirimycin which inhibited trimming of the innermost glucose residue on the transferred high mannose oligosaccharide, but did not inhibit glycosylation as measured by susceptibility of the protein to endoglucosaminidase-H and incorporation of ^{3}H-galactose and ^{3}H-fucose (Gross et al., 1983). The authors also showed that only those alpha-1-proteinase inhibitor molecules, in which oligosaccharide processing was not inhibited, were secreted, demonstrating the importance of oligosaccharide processing for the secretion of alpha-1-proteinase inhibitor.

SECTION C: HSV-INDUCED GLYCOPROTEINS

1.16 Nomenclature of the HSV glycoproteins

The rules for naming the HSV glycoproteins as discussed at the Eighth International Herpesvirus Workshop (Oxford) are as follows:-

(i) All forms of a glycoprotein arising from a particular gene shall have the same letter designation, prefixed by the small letter "g" (e.g. gB);

(ii) Unstable or stable precursor forms shall have the prefix "p" (e.g. pgB). Different precursor forms can be distinguished by adding the apparent MW ($x10^{-3}$) in parenthesis;

(iii) Newly discovered glycoproteins shall be assigned the next alphabetic letter in sequence;

(iv) The suffix "-1" or "-2" can be used to distinguish between HSV-1 and HSV-2 encoded glycoproteins (e.g. gB encoded by HSV-1 is gB-1);

(v) The terms gA and gF shall not be used.

This is because the glycoprotein originally designated gA (Spear, 1976) is now known to be antigenically related to gB (Eberle and Courtney, 1980b; Pereira et al., 1981) and the HSV-2 glycoprotein designated gF originally identified by Balachandran et al. (1981) is now known to be the genetically related counterpart of gC-1 (Para et al., 1983; Zweig et al., 1983; Zezulak and Spear, 1983, 1984b). The glycoprotein designated gC-2 by Ruyechan et al. (1979) was incorrectly mapped and thought to be the counterpart of gC-1. It has now been remapped and redesignated as gG-2 (Roizman et al., 1984) and probably corresponds to the protein originally designated g92K and mapped by Marsden et al. (1978, 1984).

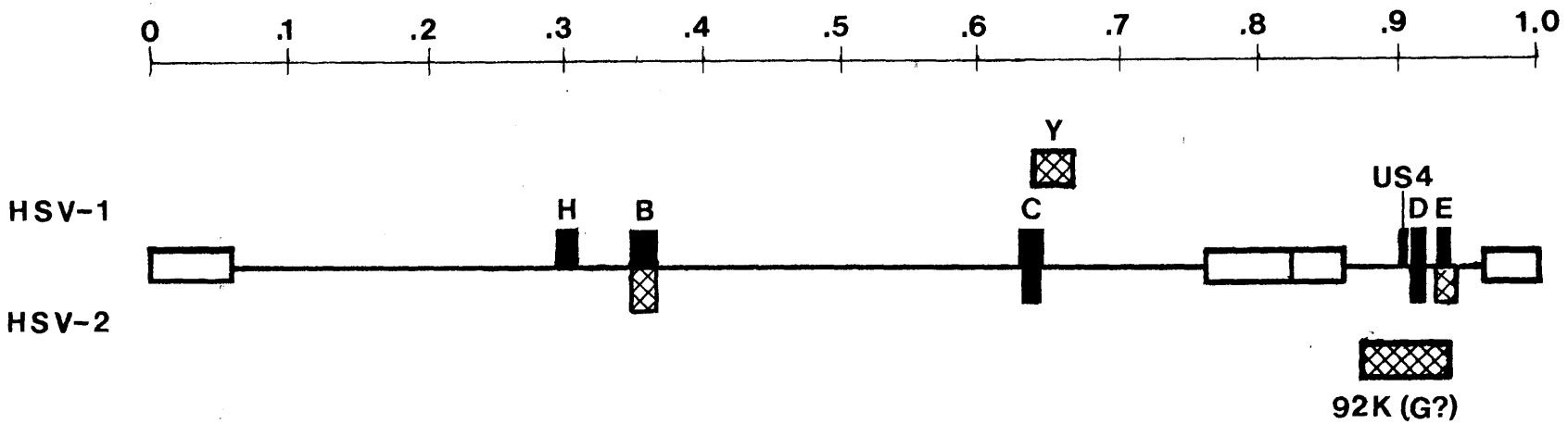
1.17 Genetic locations of the HSV glycoproteins

Seven glycoproteins have been identified in HSV-infected cells (fig. 17) - gB, gC, gD (Spear, 1976), gE (Baucke and Spear, 1979), gG (Roizman et al., 1984), g92K (Marsden et al., 1978, 1984) and gH (Buckmaster et al., 1984). The genes encoding gB, gC, gD and gE were originally mapped onto the HSV genome using HSV-1 x HSV-2 intertypic recombinants (Marsden et al., 1978; Ruyechan et al., 1979; Hope et al., 1982; Para et al., 1982b, 1983; Hope and Marsden, 1983). Lee et al. (1982a) in vitro selected mRNA to define the genome locations of gD and gE more precisely.

g92K identified by Marsden et al. (1978) in HSV-2-infected cells was shown to be distinct from gB-2, gC-2, gD-2 and gE-2 (Marsden et al., 1984). The map location of g92K overlaps the map location of gG (Roizman et al., 1984). Thus it is possible that they may be the same protein. No type-1 counterparts of g92K or gG have been identified yet.

gH was identified as a new glycoprotein by Buckmaster et al. (1984) by immunoprecipitating with monoclonal antibodies and mapping studies. Immunoprecipitation experiments using the monoclonal antibody directed against gH or the monoclonal antibody directed against a 110000 dalton glycoprotein identified by Showalter et al. (1981) showed that gH corresponds to the 110000 dalton glycoprotein (Buckmaster et al., 1984).

A glycoprotein designated gY (Palfreyman et al., 1983) was induced in cells infected with HSV-1. It has the same apparent MW as gC-1 but has a more basic isoelectric point. It is not known whether gY is antigenically related to one of the other previously identified glycoproteins, however, it has been mapped by HSV-1 x HSV-2 intertypic recombinants and lies between co-ordinates 0.64-0.67mu, which spans the region encoding gC-1 which suggests it and gC-1 may be related (H.S. Marsden, personal communication).



B penetration

C adsorption, non-essential in TC, (C-1, C3b receptor)

D adsorption

E F_c receptor, adsorption

G (92K?)

H plaque formation

FIGURE 17

Representation of the HSV genome in the prototype orientation showing the approximate map locations of the genes encoding the viral glycoproteins. The scale represents the fractional genome length (mu). Solid boxes (■) represent those genes which have been sequenced. Hatched boxes (▨) represent those genes which have not been sequenced.

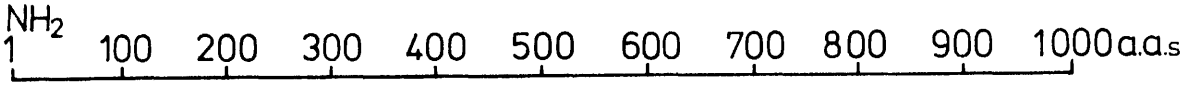
More recently, the complete DNA sequence has been obtained for the following glycoproteins: gB-1, strain F (Pellet et al., 1985) and strain KOS (Bzik et al., 1984a); gC-1, strain KOS (Frink et al., 1983); gC-2, strain G (Dowbenko and Lasky, 1984) and strain 333 (Swain et al., 1985); gD-1, strain Patton (Watson et al., 1982) and strain 17 syn+ (McGeoch et al., 1985); gD-2 from strain G (Watson, 1983) and gE-1 from strain 17 syn+ (McGeoch et al., 1985).

1.18 Primary structures of the HSV glycoproteins

Immunological approaches, using monospecific antisera or monoclonal antibodies have established that each of the glycoproteins, gB (Norrild et al., 1978a; Pereira et al., 1981; Showalter et al., 1981), gC (Zweig et al., 1983, 1984; Zezulak and Spear, 1983, 1984b), gD (Cohen et al., 1978; Pereira et al., 1980; Eisenberg et al., 1980, 1982b) and gE (Baucke and Spear, 1979; Para et al., 1982b) have antigenically related counterparts in HSV-1 and HSV-2. This suggests a degree of homology and similarity in structure between the corresponding glycoproteins of HSV-1 and HSV-2.

More precise data about their structure has come from sequencing the genes encoding the HSV glycoproteins. The important features of the predicted amino acid sequences are shown in fig. 18. It should be noted that the gC-1 sequence (Frink et al., 1983) has been amended by Draper et al. (1984a) and Dowbenko and Lasky (1984) and the revised predicted sequence is twelve amino acids (aa) shorter at the carboxy-terminus than the original. Furthermore, the carboxy terminal three amino acids are changed from ile-gly-gly-COOH to his-arg-arg-COOH.

Dowbenko and Lasky (1984) and Swain et al. (1985) estimated the homology between gC-1 and gC-2 to be 62% and 69% respectively. These homology studies predict a region in the gC-1 gene which is not present in



	(strain)		predicted mol. wt.	reference
gB-1	(KOS)	COOH(903)	100300	Bzik et. al.(1984a)
gB-1	(F)	COOH(903)	100113	Pellet et. al.(1985)
gC-1	(KOS)	COOH(523)	55064	Frink et. al.(1983)
gC-2	(333)	COOH(480)	51600	Swain et. al.(1985)
gC-2	(G)	COOH(479)	51915	Dowbenko and Lasky (1984)
gD-1	(17syn+)	COOH(394)	43344	McGeoch et. al.(1985)
gD-1	(Patton)	COOH(394)	43291	Watson et. al.(1982)
gD-2	(G)	COOH(393)	43082	Watson (1983)
gE-1	(17syn+)	COOH(550)	59090	McGeoch et. al.(1985)

FIGURE 18

Summary of the HSV-glycoprotein primary structures based on published DNA sequences (references are given in the right-hand column). Each horizontal line represents the length of the polypeptide chain, the number of amino acids in the protein is printed in parenthesis beside the carboxy (COOH) terminus. The amino (NH_2) terminus is taken as amino acid (aa) number 1. The glycoprotein represented and the strain from which the DNA was sequenced is indicated on the left-hand side. The essential features predicted for each glycoprotein are shown. These are, the signal sequence (solid boxes), membrane anchoring sequences (open boxes) and potential sites for the addition of N-linked oligosaccharides (arrows). For those glycoproteins which have been sequenced from more than one strain, conserved potential oligosaccharide addition sites are shown by dotted lines. The MW of the predicted primary amino acid sequence is also shown. The stipled boxes below the line representing gC-1 indicate sequences missing from gC-2 as predicted by Dowbenko and Lasky (1984) (D) or Swain et al. (1985) (S) (see text).

the gC-2 gene. Although both gC-2 genes (from different strains) are virtually identical (1aa addition and 1 change, strain 333 of HSV-2; Swain et al., 1985), both authors predict deletions in a different region of the gene. There is a sequence of sixty-six amino acids in gC-1 spanning the two deletions of gC-2. Of these, Swain et al. (1985) can align sixteen amino acids and Dowbenko and Lasky (1984) can align eleven amino acids. This data suggests the region predicted by Swain et al. (1985) is correct, however, the possibility exists that neither alignment is correct. This deletion is of interest since gC-1, but not gC-2, has been shown to contain a receptor for the C3b component of complement (Friedman et al., 1984).

Comparison of the predicted amino acid sequences of gD-1 and gD-2 (Watson, 1983) shows 86% homology. Eisenberg et al. (1984) have obtained the amino terminal sequence of native gD-1 (HG strain) and native gD-2 (strain Savage) by direct amino acid sequencing of the purified protein. Their data indicate that the first twenty-five amino acids of the predicted open reading frame of both serotypes are not present in the purified protein, thus identifying the site of cleavage after the signal sequence. This is in agreement with the predicted signal sequences of Watson et al. (1982), Watson (1983) and McGeoch et al. (1985).

Studies to estimate the MW of the polypeptide backbone of the HSV glycoproteins have included translation of selected mRNA, cleavage of the oligosaccharides, or inhibiting the transfer of oligosaccharides from dolichol pyrophosphate to the polypeptide. The MW of the predicted amino acid sequences can be compared with the observed MW estimated from SDS-gels. Table 4 shows this comparison and indicates the experimental approach. The predicted MWs are compatible with the observed MWs and suggests that each of the experimental approaches can be used as a reasonable estimate of the actual MW of the polypeptide backbone.

Studies investigating the number and location of the antigenic

TABLE 4

	HSV-1			HSV-2		
	Predicted MW	Observed MW	Reference	Predicted MW	Observed MW	Reference
gB	100,300 (KOS) 100,113 (F)	95,000 to 105,000	a,b,c,d,E,4,5	ND	ND	
gC	55,064 (KOS)	65,000 to 75,000	1,E,F	51,951 (G) 51,600 (333)	54,000	G
gD	43,291 (Patton) 43,344 (17^+)	46,000 to 51,000	d,2,3,4	43,082 (G)	ND	
gE	59,090 (17^+)	66,000	d,3	ND	ND	

	References	glycoprotein (observed MW)
a	Bond et al. (1982)	gB-1 (95,000)
b	Norrild and Pederson (1982)	gB-1 (98,000)
c	Kousoulas et al. (1983)	gB-1 (100,000)
d	Hope and Marsden (1983) and this study	gB-1 (105,000); gD-1 (47,000); gE-1 (70,000)
E	Wenske et al. (1983)	gB-1 (97,000); gC-1 (75,000)
F	Zweig et al. (1984)	gC-1 (65,000)
G	Zezulak and Spear (1983)	gC-2 (54,000)
1	Frink et al. (1983)	gC-1 (69,000)
2	Watson et al. (1982)	gD-1 (46,000)
3	Lee et al. (1982a)	gD-1 (51,000); gE-1 (66,000)
4	Palfreyman et al. (1983)	gB-1 (105,000); gD-1 (47,000)
5	Reefield and Knipe (1984)	gB-1 (99,000)

TABLE 4

Comparison of the MWs of the predicted primary amino acid sequence (see fig. 18) of gB, gC, gD and gE of HSV-1 and HSV-2 strains with the observed MW of glycoproteins under conditions in which glycosylation was inhibited. The observed MWs were estimated by SDS-PAGE analysis of cells infected with HSV after treatment with tunicamycin (small letters, e.g. a), solubilised extracts of infected cells treated with endoglycosidases (capital letters, e.g. E) or in vitro translation of mRNA (numbers). The letters or numbers indicate the reference in which the data is given.

determinants of HSV-glycoproteins have been carried out. Ten epitopes of gD have been identified (Eisenberg et al., 1982a; Dietzschold et al., 1984) using limited proteolysis and observing, by SDS-PAGE, which fragments remained bound to monoclonal antibodies. Three of these epitopes are HSV-2-specific, three are HSV-1-specific and four are type-common (Dietzschold et al., 1984; Eisenberg et al., 1985). Of these ten epitopes, four of them are destroyed when the protein is denatured, suggesting that they depend on the tertiary structure of gD. Eisenberg et al. (1985) determined that the four epitopes susceptible to denaturation lie within the first 260 amino acids of the mature protein since antibodies directed against these four epitopes reacted with truncated gD (residues 1-260). The remaining six epitopes were recently located (fig. 19) using synthetic peptides (Eisenberg et al., 1984, 1985; Dietzschold et al., 1984).

The antigenic determinants in gC-1 have also been studied. Marlin et al. (1985) identified nine epitopes distributed between two distinct regions of gC-1 (fig. 19). These authors used a series of antigenic variants of HSV-1 (designated monoclonal antibody resistant (mar) mutants), each of which express an antigenically altered form of gC-1 and which are resistant to neutralisation with at least one member of a panel of gC-specific, neutralising monoclonal antibodies. The epitopes were grouped into antigenic sites by evaluating the pattern of neutralisation observed with the panel of monoclonal antibodies.

1.19 HSV-glycoprotein synthesis

1.19.1 Kinetics

The synthesis of some of the HSV glycoproteins have been investigated. Fig. 20 represents a summary of this data. Balachandran et al. (1982b) determined the kinetics of synthesis of HSV-2 glycoproteins by immunoprecipitation with monoclonal antibodies directed against gB, gC,

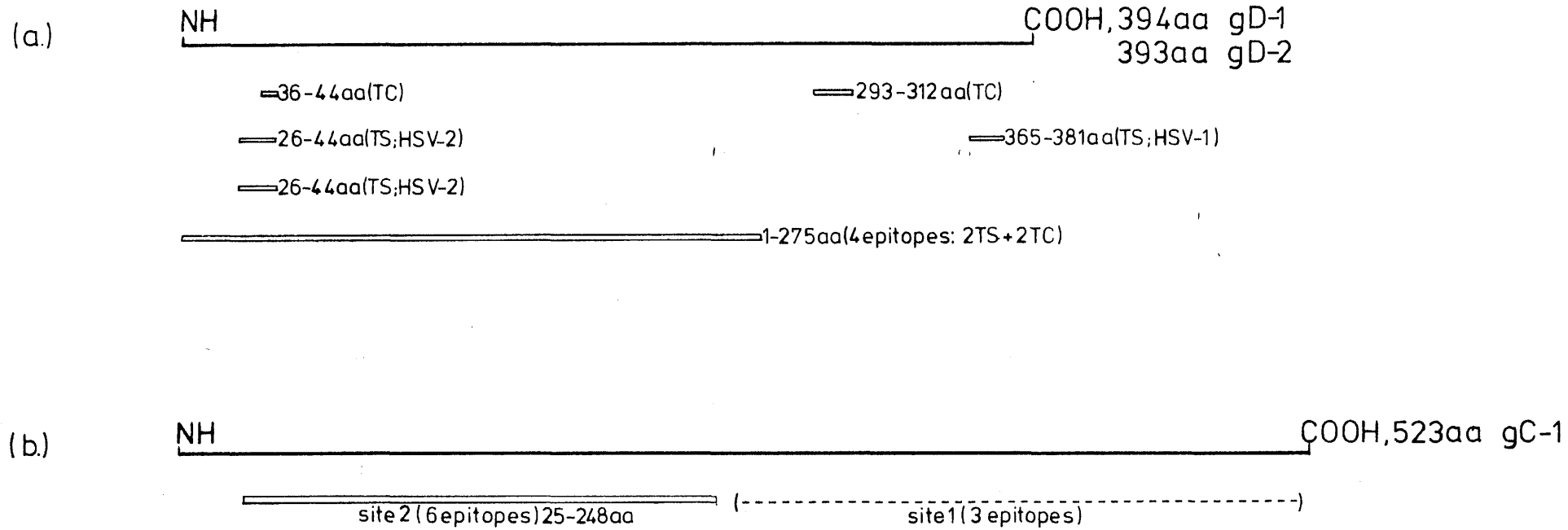
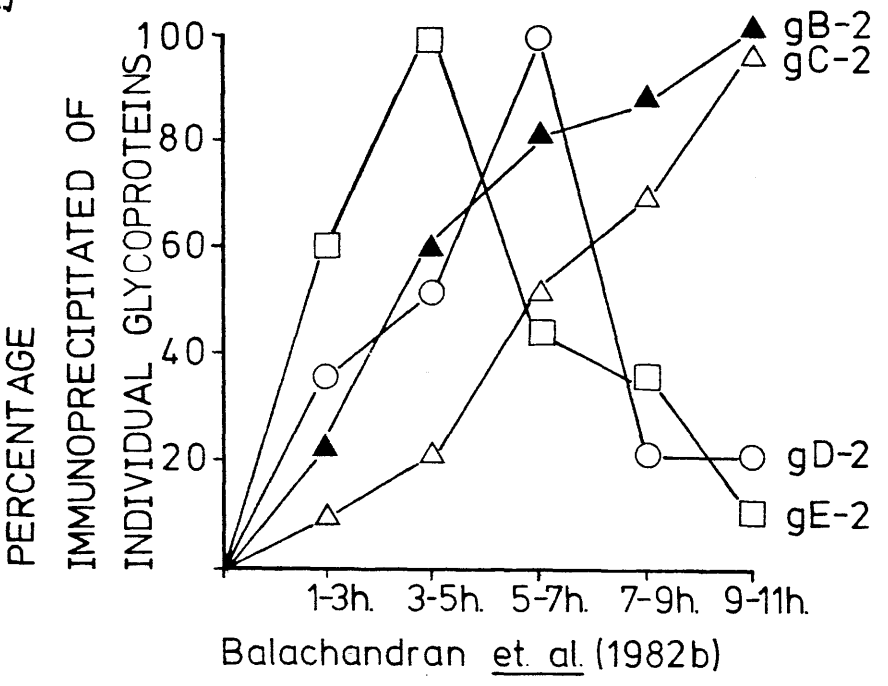

(a.)
NH
COOH, 394aa gD-1
393aa gD-2
36-44aa(TC)
293-312aa(TC)
26-44aa(TS;HSV-2)
365-381aa(TS;HSV-1)
26-44aa(TS;HSV-2)
1-275aa(4epitopes: 2TS+2TC)
(b.)
NH
COOH, 523aa gC-1
site 2 (6 epitopes) 25-248aa
site 1 (3 epitopes)

FIGURE 19

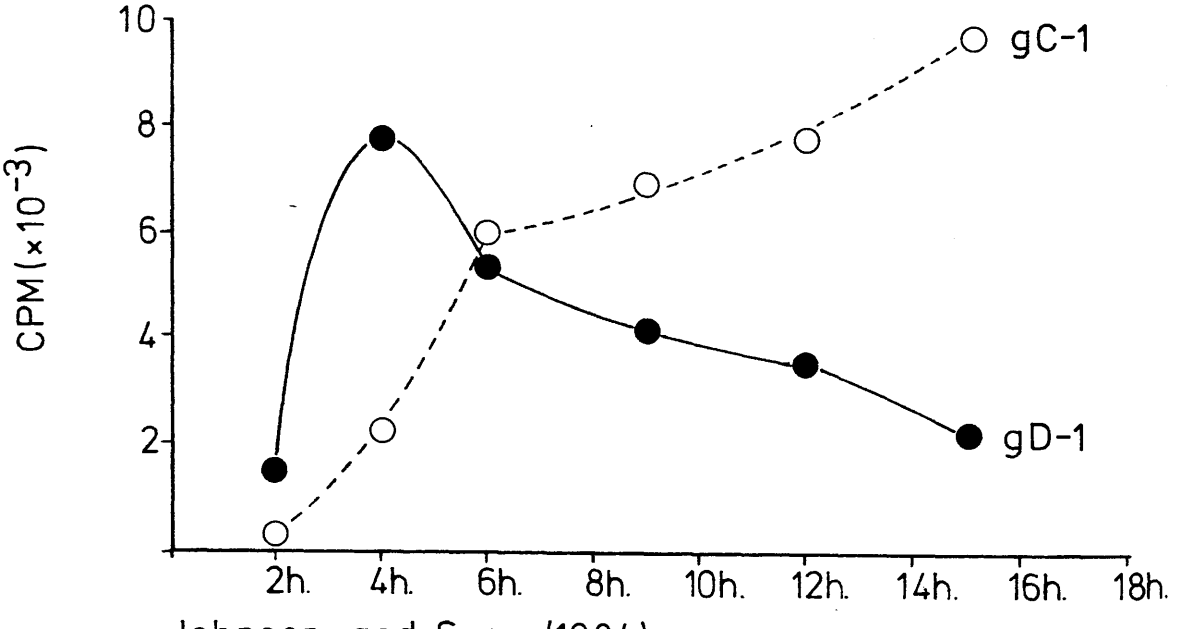
Summary of the location of epitopes in (a) glycoprotein D, (b) glycoprotein C of HSV-1. The long horizontal lines represent the length of the predicted polypeptide chain, the number of amino acids (aa) in each protein is printed at the carboxy (COOH) terminus. The amino (NH_2) terminus is taken as amino acid number 1. The open boxes (▭) represent that part of the polypeptide chain which contains a particular epitope. The broken line in parenthesis indicates that this site was located on the basis that monoclonal antibodies immunoprecipitated wild-type gC-1, but did not immunoprecipitate truncated forms of gC-1. An antigenic site (e.g. site 1 in gC-1) may contain more than one epitope (Yewdell and Gerhard, 1981). A (TC) or (TS) after an epitope denotes whether it is type-common or type-specific, respectively.

(a)



Balachandran et. al. (1982b)

(b.)



Johnson and Spear (1984)

FIGURE 20

Kinetics of synthesis of HSV glycoproteins:

(a) Immunoprecipitated HSV-2 polypeptides were excised from gels and counted in a liquid scintillation counter. The amount of radiolabelled glycoproteins precipitated relative to the total amount of [^{35}S]-methionine incorporated into TCA-precipitable material during each 2h pulse were then calculated and expressed as percentages of the values corresponding to maximum synthesis of each glycoprotein.

(b) Quantitation of gC-1 and gD-1 synthesised at various times after infection with HSV-1. Immunoprecipitated polypeptides were excised from gels and dissolved in 2% periodic acid, and then the radioactivity was counted.

gD and gE (fig. 20a). All glycoproteins were synthesised as early as 1-3h. Synthesis of gE-2 and gD-2 increased till 3-5h and 5-7h respectively, but decreased thereafter. Synthesis of gB-2 and gC-2 increased throughout the infectious cycle.

The kinetics of synthesis of gD-1, gC-1 (Fig. 20b) (Johnson and Spear, 1984) and gE-1 (this thesis), are comparable with those of gD-2, gC-2 and gE-2 respectively (Balachandran et al., 1982b).

1.19.2 Regulation

The HSV proteins are under temporal regulation (see Section 1.8.1). The synthesis of the HSV-1 glycoproteins have been studied with respect to their requirements for prior viral DNA synthesis. Experiments with the DNA inhibitor phosphonoacetic acid (PAA) suggest that gC-1 is in the gamma (late) regulated class (Peake et al., 1982), while gB-1 and gD-1 do not fit neatly into the scheme of Honess and Roizman (1974, 1975). Wagner (1985) suggested that gB-1 and gD-1 be termed beta-gamma because synthesis of gB-1 and gD-1 is initiated before DNA synthesis, but maximum rates of synthesis are not reached till after DNA synthesis. However, this beta-gamma class should probably be subdivided since gB-1 is made in increasing amounts throughout infection while gD-1 synthesis peaks 5-7h after infection. PAA prevents the synthesis of gC-1 mRNA and supports its classification as a late gene (Peake et al., 1982).

The regulation of expression of gD-1 has been studied extensively (Gibson and Spear, 1983; Everett, 1983, 1984a, b; Everett and Dunlop, 1984). All the DNA sequences necessary for regulated expression of transcription of gD-1 mRNA lie within 83bp of the RNA start site (Everett, 1983). Earlier studies established that the product of IE gene 3 ($V_{MW}175$) was essential for early gene expression (Preston, 1979a; Watson and Clements, 1980).

Johnson and Spear (1984) presented evidence for regulation of the synthesis of gD-1 at the translational level. They showed, by transfer of mRNA extracted from infected HEp-2 cells to nitrocellulose followed by hybridisation with cloned HSV-1 DNA fragments, that gD-1 mRNA accumulates at times when gD-1 polypeptide synthesis declines. However, this result contrasts with data by Johnson, A.P., MacLean, C., Marsden, H.S., Dalziel, R.G. and Everett, R.D. (manuscipt in preparation) who show, using quantitative S1 mapping, that gD-1 mRNA extracted from infected BHK cells declines at the same time as gD-1 polypeptide synthesis. Whether these contrasting results are due to the different techniques used and/or the cell line, remains to be tested.

Comparison of the gD-1 promoter region with the analogous regions in other glycoprotein genes (fig. 21a) shows that there are no sequences present at equivalent positions in all the genes. However, G-rich sequences can be identified in all of the promoters and AC-rich regions can be identified in most of the promoters at approximately the same locations. Similarly, a comparison of the DNA sequences around the 3' terminus of the mRNAs (fig. 21b) identifies the polyadenylation signal (Proudfoot and Brownlee, 1974, 1976) about 20bp before the 3' terminus and a GT-rich sequence about 30bp beyond the AATAAA signal (McLauchlan et al., 1985) thought to play a role in the processing of mRNA 3' terminus.

Mapping of the mRNAs transcribed from the HSV-glycoprotein genes have identified families of mRNAs which share 3' termini. These include gC-1 (Frink et al., 1983), gC-2 (Draper et al., 1984b), gD-1 (Watson et al., 1983; Ikura et al., 1983; Rixon and McGeoch, 1985) and gE-1 (Rixon and McGeoch, 1985), but not the mRNAs of gB-1 (Bzik et al., 1984a; Rafield and Knipe, 1984). The possibility exists that the function of the translation products from these mRNA families relates in some way,

(A)

```
                -100        -90        -80        -70        -60        -50        -40        -30        -20        -10         1
gD-1  1,2  CCATACCGACCACACCGACGAATCCCCCAAGGGGGAGGGGCCATTTTACGAGGAGGAGGGGTATAACAAAGTCTGTCTTTAAAAAGCAGGGGTTAGGGAGTT
gD-2  3    ACCACATAGTCAACCAAATCACCCCCAGAGGGGAGGTTCCATTTTTACGAGGAGGAGGAGTATAATAGAGTCTTTGTGTTTAAAACCCGGGGTCGGTGTGGT
gC-1  4    CATTAGTCCCGAAGACCGCCGGTGTGTGATGATTTGCGCCATAACACCCAAACCCCGGATGGGGCCCGGGTATAAATTCCGGAAGGGGACACGGGCTACCTA
gC-2  5    GATTTCGCCCTACCGCTCCGATCCCCGGGGGGAGGGGGGAAGGAAATGGGGGCGGGGGTGCCGTGGACGGGTATAAAGGCCAGGGGGGCAGGCGGGCCCATCA
gB-1  6,7  CCGTCAGGGAATTTCAGAGGTTTTACTGTTTTGACGGCATTTCCGGAATAACGCCCACTCAGCGCGCCGCCTGGCGATATATTCGCGAGCTGATTATCGCCA
gE-1  1    CGGAAACCAAAGAAGGAAGCAAAGAATGGATGGGAGGAGTTCAGGAAGCCGGGGAGAGGGCCCGCGGCGCATTTAAGGCGTTGTTGTGTTGACTTTGCCTCT
```

(B)

```
                1          10         20         30         40         50         60         70         80
gD-1  1    AATAAACCGGTATTATTTACCTATATCCGTGTATCTCGATTTCTTTCCCCCCCTCCCCGGAAACCAAAGAAGGAAGCAAA
gC-1  4    AATAAACACACAATCACGTGCGATAAAAAGAACACGCGGTCCCCTGTGGTGTTTTTGGTTATTTTTATTAAATCTCGTCG
gC-2  5    AATAAACAGCTAATTGCGTACGACAAACCATGCGGAACTCGCTGTTTTTTTTTCTCTGTTTGTTACTTTTTATTGAAAAC
gB-1  6    AATAAAAACCACGGGTGTTAAACCGCATGCGCATCTTTTGGTTTTTTTGTTTGGTCAGCCTTTTGTGTGTGTGGGAAGAA
gB-1  7    AATAAAAACCACGGGTGTTAAACCGCATGTGCATCTTTTGGTTTGTTTGTTTGGTACGCCTTTTGTGTGTGTGGGAAGAA
gE-1  1    ATTAAATTGGGTTCGATTGGCAATGTTGTCTCCCGGTTGAGATTTTTGGGTGGGTGGGGAGTGGGTGGGTGGGGAGTGGG
```

FIGURE 21

DNA sequences (a) upstream of the 5' end, and (b) downstream of glycoprotein mRNAs. All sequences are in the 5'-3' orientation. (a) shows the 85 bases upstream of the transcription initiation site. The TATA box (Breathnach and Chambon, 1981; Benoist et al., 1980; Efstratiadis et al., 1980) for each sequence is underlined. Also indicated by a dashed line are those sequences (which are similar to the sequence elements thought to be important for the activation of transcription of the gD-1 gene (Everett, 1983)) which may be important for the activation of transcription of that gene. (b) shows the 80 bases downstream from the polyadenylation signal AATAAA or ATTAAA (underlined) (Proudfoot and Brownlee, 1974, 1976). The 3' end of the mRNAs (- - - -:) where known, are indicated and the GT-rich sequence (underlined) which are thought to play a role in post-transcriptional processing of the 3' terminus (McLauchlan et al., 1985) are also underlined.

The numbers superscripted above the designated glycoproteins are the codes for the references (cited below) from which the sequence data were taken:

1 McGeoch et al. (1985)

2 Watson et al. (1982)

3 Watson (1983)

4 Frink et al. (1983)

5 Swain et al. (1985)

6 Bzik et al. (1984a)

7 Pellet et al. (1985)

not yet established, to the function of the glycoproteins. An example of two 3' co-terminal mRNAs whose gene products are functionally related are the 5.0kb (kilobase) and 1.2kb mRNA located between 0.56 and 0.60mu on the HSV-1 genome (McLauchlan and Clements, 1982, 1983) (see Section 1.7.1). These two mRNAs encode the $V_{MW}136'$ (143) and $V_{MW}38$ polypeptides which associate to form the ribonucleotide reductase complex (Frame et al., 1985).

1.20 Processing of the HSV-glycoproteins

Evidence is accumulating that HSV-glycoproteins are processed in a similar manner to that described in Section B. That is, N-linked oligosaccharides are added "en bloc" from dolichol pyrophosphate to the nascent polypeptide chain being synthesised on membrane bound ribosomes in the rough ER. Both processing of the N-linked and addition of O-linked oligosaccharides take place in the ER and the Golgi complex before the glycoproteins reach their destination in the infected cell membrane.

1.20.1 Cleavage of signal sequences

DNA sequence data of the genes encoding the HSV glycoproteins, described in Section 1.18, identified features characteristic of integral membrane proteins, such as signal sequences, which are normally cleaved from the protein during maturation (see Section 1.10.1) and anchor sequences (see Section 1.10.2). Amino acid sequence data of the amino-terminus of gD-1 and gD-2 (Eisenberg et al., 1984) indicate that the first twenty-five amino acids (which encompass the putative signal sequence) predicted from the DNA sequence of these genes, have been cleaved off during processing. It has not yet been established whether similar cleavages occur during processing of the other HSV glycoproteins.

1.20.2 Addition of N-linked oligosaccharides

Evidence that the HSV glycoproteins contain N-linked oligosaccharides is summarised in Table 5. Synthesis of both the precursor and the mature forms of gB-1, gC-1, gD-1, gE-1 and gG-2 is inhibited when infected cells are grown in the presence of tunicamycin (Pizer et al., 1980; Bond et al., 1982; Norrild and Pederson, 1982; Kousoulas et al., 1983; Balachandran and Hutt-Fletcher, 1985; Hope and Marsden, 1983; this thesis), suggesting that N-linked oligosaccharides are added "en bloc" from dolichol pyrophosphate to asparagine. Furthermore, pgB-1, pgC-1, pgD-1, pgE-1 and pgG-2, but not the mature forms of these species with the exception of gB-1, are sensitive to endo-H (an enzyme which cleaves high mannose, but not complex type oligosaccharides).

Compton and Courtney (1984a) presented evidence compatible with the model in which N-linked oligosaccharides are added onto the nascent polypeptide being synthesised on the rough ER which buds from the nuclear membrane. These authors fractionated HSV-1-infected cells and showed that the high mannose oligosaccharide-containing precursors are mainly associated with the nuclear fraction, while the cytoplasmic fraction contained mainly mature glycoproteins which were endo-H insensitive. This result suggests that the HSV glycoproteins acquire their N-linked oligosaccharides at a site at or near the nuclear membrane.

1.20.3 Processing of the N-linked oligosaccharides

Evidence which suggests that the N-linked oligosaccharides attached to HSV glycoproteins are processed in a similar manner to that described in Section 1.11.2, comes from studies using drugs which block processing or cell lines which are deficient in glycosyltransferases. Table 6 is a summary of the results from several investigators who have examined processing of the HSV glycoproteins. It was observed by using either cells

TABLE 5

Glycoprotein	pgB-1	gB-1	pgC-1	gC-1	pgD-1	gD-1	pgE-1	gE-1	pgG_1-2 (120K)	pgG_2-2 (74K)	gG-2 (108K)	gY-1
Synthesis inhibited by tunicamycin	ck YES	abcdk YES	ND	YES	ck YES	abcdk YES	k YES	k YES	j YES	j YES	j YES	k YES
Sensitive to endo-H	efghi YES	gi YES	eghi YES	eghi NO	efi YES	ei NO	i YES	i NO	j YES	j YES	NO	ND
Sensitive to NAGO	i NO	i YES	i NO	i YES	i NO	i YES	ND	ND	ND	ND	ND	ND

References

a Pizer et al. (1980)
b Bond et al. (1983)
c Norrild and Pederson (1982)
d Kousoulas et al. (1983)
e Serafini-Cessi and Campadelli-Fiume (1981)
f Person et al. (1982)
g Wenske et al. (1980
h Compton and Courtney (1984a)
i Johnson and Spear (1983)
j Balachandran and Hutt-Fletcher (1985)
k This thesis; Hope and Marsden (1983)
ND No data

TABLE 5

Susceptibility of the mature HSV glycoproteins or their precursors to inhibition of synthesis by tunicamycin and cleavage by endoglycosaminidase-H (endo-H) or N-acetylgalactosaminyl oligosaccharidase (NAGO). The references from which data are taken are indicated above the result: YES for susceptible and NO for not susceptible.

TABLE 6

Method by which glycoprotein synthesis is blocked	GLYCOPROTEINS				
	B^a	C^{abcd}	D^{abc}	E^a	G^d
Cells infected in the presence of monensin	pgB-1	pgC-1	pgD-1	pgE-1	pgG-2 (110K)
Infection of ricin-resistant cells	pgB-1^e	pgC-1^e	pgD-1^e	ND	ND

References

a Johnson and Spear (1982)
b Johnson and Spear (1983)
c Wenske et al. (1982)
d Balachandran and Hutt-Fletcher (1985)
e Campadelli-Fiume et al. (1982)
ND Not Done

TABLE 6

Stage at which the processing of the HSV glycoproteins is blocked when (i) BHK cells are infected with HSV-1 or HSV-2 in the presence of monensin, or (ii) a ricin-resistant cell line (deficient in N-acetyl-glucosamine transferase I) is infected with HSV-1.

infected with HSV-1 in the presence of monensin (Johnson and Spear, 1982, 1983; Wenske et al., 1982; Balachandran and Hutt-Fletcher, 1985) an ionophore which blocks transport of glycoproteins to the cell surface membrane (see Section 1.12), or an HSV-1-infected ricin-resistant cell line which is deficient in N-acetylglucosamine (glcNAc) transferase I (Campadelli-Fiume et al., 1982) (an enzyme which is essential for the conversion of high mannose to complex type oligosaccharides, see Section 1.11.2) that the endo-H sensitive precursor species of the glycoproteins accumulate, with a concomitant decrease in amounts of the mature glycoproteins.

More recently, processing has been investigated in greater detail. Serafini-Cessi et al. (1984b) showed that oligosaccharides cleaved by endo-H from pgC-1 contained high mannose oligosaccharides with the carbohydrate composition of the oligosaccharides varying from mann(9) glcNAc (nine mannose and one N-acetylglucosamine residues) to mann(5) glcNAc. Serafini-Cessi et al. (1983a) used an HSV-1-infected ricin-resistant cell line deficient in glycosyltransferases of the Golgi complex which add terminal sugars to complex oligosaccharides, to show a reduction in bi-antennary (two branched) and tri-antennary oligosaccharides of HSV glycoproteins.

1.20.4 O-linked oligosaccharides

Evidence showing that the HSV glycoproteins also contain O-linked oligosaccharides has been obtained by a number of investigators. Studies using helix pomatia and soybean lectins which bind galNAc (Goldstein and Hayes, 1978; Hammerstrom et al., 1977), a sugar not yet found in N-linked oligosaccharides (Kornfeld and Kornfeld, 1976) have been used to suggest the presence of O-linked oligosaccharides on HSV glycoproteins. Olofsson et al. (1981a) showed that these lectins bind a glycoprotein from

HSV-1-infected cells, but not from uninfected cells, suggesting that at least one of the HSV-1 glycoproteins contained O-linked oligosaccharides. This suggestion was supported by the observation (Olofsson et al., 1981b) that the bound glycoprotein was sensitive to mild alkaline hydrolysis, a treatment known to remove O-linked oligosaccharides, but not N-linked oligosaccharides from the polypeptide chain. Olofsson et al. (1981b) used a monospecific antiserum to show that the lectin binding HSV-1 glycoprotein was gC-1.

More recently, Olofsson et al. (1986) have shown that helix pomatia and soybean lectins bind a glycoprotein from HSV-2-infected cells which is immunoprecipitable by the monoclonal antibody AP1, has an apparent MW of 130000 and maps between co-ordinates 0.892-0.924mu on the HSV-2 genome. The characteristics of this lectin binding protein is compatible with it being the g92K glycoprotein (see Sections 3.10 and 3.13 described in this thesis).

Johnson and Spear (1983) presented direct evidence that gB-1, gC-1 and gD-1 contained O-linked oligosaccharides. These authors showed that those glycoproteins when treated with N-acetylgalactosaminyl oligosaccharidase (NAGO) which cleaves O-linked oligosaccharides (see Section 1.13), yield polypeptides which co-migrate with pgB-1, pgC-1 and pgD-1 respectively. These precursors were not sensitive to this enzyme (Table 5) suggesting that the increase in MW from the precursor form to the mature form of the glycoprotein is due entirely to the addition of O-linked oligosaccharides. Furthermore, these authors showed that the addition of O-linked oligosaccharides to HSV glycoproteins is a post-translational event, by infecting cells in the presence of monensin (see Section 1.12). The precursors, pgC-1 and pgD-1, which accumulated under these conditions are not sensitive to NAGO suggesting that the O-linked oligosaccharides are added to HSV glycoproteins in the Golgi complex and

after the addition of N-linked oligosaccharides.

1.20.5 Evidence for proteolytic cleavage

There is evidence that at least two HSV glycoproteins undergo major proteolytic cleavage, gE-1 (this thesis, see Section 3.8.7) and g92K (Balachandran and Hutt-Fletcher, 1985).

Pulse-chase experiments by Balachandran and Hutt-Fletcher (1985) showed that a polypeptide of apparent MW of 110000 (110K) (determined on gels cross-linked with DATD) is processed to an endo-H sensitive 120K precursor then matures into a glycoprotein of apparent MW 108K which the authors designated gG-2. However, since the experiments were performed with the monoclonal antibody AP1 which is directed against g92K (Marsden et al., 1984; this thesis, Section 3.10) and not the monoclonal antibody H966 with which Roizman et al. (1984) defined gG-2, the designation of their glycoprotein as gG and not g92K, although probably correct, must be regarded as tentative.

Balachandran and Hutt-Fletcher (1985) also presented evidence suggesting that the 108K glycoprotein undergoes proteolytic cleavage during its maturation. Polypeptides of apparent MW 74K (smaller than the 110K primary translated product) and 120K, which are both endo-H sensitive, were immunoprecipitated from cells infected with HSV-2 in the presence of monensin using a monoclonal antibody with the same specificity as AP1, suggesting that the 74K is the result of proteolytic cleavage of the 120K precursor and is the precursor to the 108K glycoprotein.

The biological consequences of proteolytic cleavage of gG-2 and gE-1 are not yet known, but are discussed in Section 4.7.

1.21 Analysis by 2-D PAGE

Glycosylation of the HSV glycoproteins involves at least the addition of fucose, sialic acid (Honess and Roizman, 1975) and mannose (Eisenberg et al., 1979). Using the technique of 2-D PAGE, described by O'Farrell et al. (1977), it was originally observed that gB-1 (Haarr and Marsden, 1981), gC-1 (Cohen et al., 1980) and gD-1 (Cohen et al., 1980; Haarr and Marsden, 1981) were generated by at least 16, 15 and 12 discrete steps respectively and that these steps were due, in part, to the addition of sialic acid. These steps also involve MW and charge differences. Evidence presented in Section 1.20 suggests that differences in MW from the precursor form to the mature glycoprotein are due to both the addition of O-linked oligosaccharides and processing of N-linked oligosaccharides. Palfreyman et al. (1983) have shown that gB-1, gC-1 and gD-1 also undergo processing involving MW increases without any positive charge increase which probably are due to the transfer of the high mannose oligosaccharides from dolichol pyrophosphate to asparagine on the polypeptide chain. Furthermore, each of the polypeptide species undergoing MW increases only (for gB-1 and gD-1 at least) can also undergo increases of positive charge without any increase in MW and this probably is due to the processing of the N-linked oligosaccharides from high mannose to complex type as described in Section 1.11.2. In addition, Palfreyman et al. (1983) showed that during synthesis of gB-1, two mature species, gB118 and gB122, are generated. gB118 and gB122 respectively are likely to be the antigenically related species gA and gB (Eberle and Courtney, 1980b) identified by one-dimensional PAGE and originally thought to be distinct glycoproteins (Spear, 1976). The large number of steps observed on 2-D gels in the processing of gB-1 is perhaps a consequence of each of the oligosaccharides on the nine potential N-glycosidic sites on gB-1 (Bzik et al., 1984a) undergoing multiple processing steps.

1.22 Functions of HSV glycoproteins

Table 7 lists functions or activities with which the HSV glycoproteins have been associated.

1.22.1 Neutralisation

Early studies established that at least some of the HSV glycoproteins are expressed on the surfaces of the membranes of both virions and infected cells (Olshevsky and Becker, 1972; Heine et al., 1972; Spear, 1976; Baucke and Spear, 1979) and would therefore be expected to be targets for the host immune response.

Preparations of individual viral glycoproteins isolated by excising the relevant polypeptides from SDS-polyacrylamide gels have been used to induce the production of neutralising antibodies by injection into rabbits (Table 8). Furthermore, it has been shown that mice immunized with gC-1 purified by hydroxylapatite chromatography (Schrier et al., 1983) or immunoaffinity purified gB-1 or gC-1 (Roberts et al., 1985) or gD-1 (Chan, 1983; Lasky et al., 1984; Long et al., 1984; Paoletti et al., 1984; Weis et al., 1983; Eisenberg et al., 1982b) are protected from a lethal HSV infection.

Monoclonal antibodies directed against the HSV glycoproteins have been reported to have neutralising activity (Table 8). Also, it has been shown that mice immunized with monoclonal antibodies directed against gD-1 or gD-2 (Balachandran et al., 1982a; Dix et al., 1981; Kapoor et al., 1982; Sethi, 1983), gC-1 (Sethi, 1983), gB-2 (Balachandran et al., 1982a), and gE-2 (Balachandran et al., 1982a) are protected from a lethal HSV infection.

Rector et al. (1984) have shown that certain monoclonal antibodies against gB-1 or gD-1 do not neutralise, but can protect against ocular HSV-1 infections.

TABLE 7

Function	HSV GLYCOPROTEINS					
	gB	gC	gD	gE	gG	gH
Neutralisation	√	√	√	√	√	√
Adsorption	√	√	√			
Penetration	√	√				
Cell fusion (syn)	√		√			
Fc-receptor	-	-	-	√	-	-
C3b receptor	-	gC-1	-	-	-	-

TABLE 7

Functions of the HSV glycoproteins. Symbols used:

√	indicates that evidence has been presented (see text for references) that the glycoprotein may be involved in a particular function;
-	tested and found not to be involved;
gC-1	indicates that the C3b receptor is specific for gC of HSV-1 (and not HSV-2);
no symbol	indicates that the glycoprotein has not been implicated in the function but could possibly be.

TABLE 8

	Neutralising antibodies produced after immunizing with purified HSV-glycoproteins	Neutralising monoclonal antibodies	
		Type-specific	Type-common
gB-1	$\surd^{de}$		$\surd^{gkt}$
gB-2	NT		
gC-1	$\surd^{em}$	$\surd^{fglt}$	$\surd^{p}$
gC-2	$\surd^{o}$	$\surd^{ks}$	
gD-1	$\surd^{abcdi}$	$\surd^{h}$	$\surd^{fghknr}$
gD-2	$\surd^{i}$	$\surd^{h}$	
gE-1	$\surd^{j}$		
gE-2	NT	$\surd^{k}$	
gG-2	NT	$\surd^{ks}$	
gH-1	NT	$\surd^{q}$	$\surd^{g}$

TABLE 8

Neutralising antibodies isolated so far. Symbols:

NT indicates not tested;

blank space indicates that no monoclonal antibodies have been isolated for this particular class;

√ indicates that antibodies have been produced;

The post-scripts above each tick (√) designate the reference from which the data was obtained - see below:

a Watson and Wildy (1969)

b Cohen et al. (1972,1978)

c Honess and Watson (1974)

d Powell et al. (1974)

e Eberle and Courtney (1980a)

f Pereira et al. (1980)

g Showalter et al. (1981)

h Eisenberg et al. (1982a)

i Eisenberg et al. (1982b)

j Para et al. (1982a)

k Balachandran et al. (1982b)

l Holland et al. (1982a)

m Schrier et al. (1983)

n Weis et al. (1983)

o Zezulak and Spear (1983)

p Zweig et al. (1983)

q Buckmaster et al. (1984)

r Long et al. (1984)

s Balachandran and Hutt-Fletcher (1985)

t Roberts et al. (1985).

Thus, all the known HSV glycoproteins can induce the production of antibodies that either neutralise virions and/or can aid in the protection of the recipient against an HSV infection.

1.22.2 Adsorption

The adsorption of HSV onto cells has been studied by Johnson et al. (1984) using virosomes. Virosomes are composed of a lipid bilayer in which are inserted viral glycoproteins extracted from virion envelopes. The authors presented evidence showing that monoclonal antibodies directed against gB-1, gC-1 and gD-1 inhibited the adsorption of virosomes onto HEp-2 cells. gB-1 antibodies were more effective than gC-1 or gD-1 antibodies at inhibiting adsorption. Furthermore, virosomes depleted of gB-1 or gC-1 did not adsorb onto cells as efficiently as did virosomes containing these glycoproteins.

The evidence of Johnson et al. (1984) suggests that at least one of the HSV glycoproteins, gB-1, gC-1 or gD-1, or perhaps a combination of these glycoproteins may play a role in adsorption of HSV onto cells. This work does not rule out the possibility that, as yet, unrecognised glycoproteins may also play a role in this function.

1.22.3 Penetration

Electron microscope studies (Morgan et al., 1968) suggest that penetration of HSV occurs by fusion of the virion envelope with the cell surface membrane. Evidence supporting this hypothesis comes from experiments with the HSV-1 mutants, tsB5 (Sarmiento et al., 1979) and tsJ12 (Little et al., 1981). Neither of these mutants synthesise mature gB-1 at the NPT. Virions produced at the NPT in mutant-infected cells can adsorb onto cells, but have low infectivity. Their low infectivity could be significantly enhanced by treating the "infected" cells with polyethylene

glycol, an agent known to promote membrane fusion (Pontecorvo, 1975). These authors concluded that gB plays a role in penetration and therefore is essential for infectivity.

DeLuca et al. (1982) constructed HSV-1 x HSV-1 intratypic recombinants by marker rescue and marker transfer cotransfection procedures using intact DNA from one HSV-1 strain and recombinant plasmids containing portions of the EcoRI restriction fragment F from another HSV-1 strain. The EcoRI fragment F (map co-ordinates 0.315-0.412mu) contains the gB-1 gene. The authors used these recombinants to map a locus on the HSV-1 genome that affects the rate of penetration of HSV-1 into cells. This locus lies between the 2 loci of tsB5 which determine the syn phenotype and the ts phenotype. The mapping data of DeLuca et al. (1982) and the nucleotide sequence data by Bzik et al. (1984a) has located the mutations determining the syn and ts phenotypes in the structural gene for gB-1. Therefore the locus determining the rate of entry also lies in gB-1.

Several investigators have used a Rous Sarcoma Virus transformed cell line (XC cells) to study the replication of HSV. Docherty et al. (1973) and Campbell et al. (1974) presented evidence that penetration of HSV-1 did not occur in this cell line, whereas Garfinkle and McAuslan (1973) and Padgett et al. (1978) found that it did. A possible explanation for this apparent contradiction of results was suggested by Epstein and Jaquemont (1983) who showed that the stage at which replication is blocked in XC cells, depends on the strain of virus used. Epstein et al. (1984) used HSV-1 x HSV-1 intratypic recombinants isolated by Ruyechan et al. (1979) to map a locus on the HSV-1 genome affecting the penetration of virions into XC cells to between 0.70 and 0.83mu. The fact that these co-ordinates overlap a region on the HSV-1 genome containing a locus, identified and designated Cr by Ruyechan et al. (1979) who

suggested that Cr controls the synthesis or accumulation of gC-1, led Epstein et al. (1984) to propose that gC-1 may control penetration of HSV-1 into XC cells by negatively modulating the gB-1 promoted fusion between host cell and virion membranes. However, it should be noted that data from marker transfer and marker rescue experiments using cloned DNA fragments (Pogue-Geile et al., 1984) show that the mutation responsible for the gC$^-$ phenotype of MP, one of the HSV-1 strains from which the intratypic recombinants of Ruyechan et al. (1979) was derived, is located between co-ordinates 0.621 and 0.645mu which contains the structural gene for gC-1 and therefore places the existence of the Cr locus in doubt.

1.22.4 Cell fusion (syn phenotype)

Although clinical isolates of HSV usually do not exhibit the capacity to fuse infected cells, isolation of fusion-inducing strains from patients has occasionally been reported (Terni and Roizman, 1970). However, fusion-inducing strains of HSV can be isolated after passage of the virus in tissue culture (Hoggan and Roizman, 1959; Nii and Kamahara, 1961; Roizman, 1962; Ejercito et al., 1968; Brown et al., 1973; Timbury et al., 1974; Cassai et al., 1975, 1976; Zezulak and Spear, 1983). The fusion-inducing strains are brought about by spontaneous non-lethal mutations in the HSV genome. The phenotype of such mutations is designated syn (Enders and Peebles, 1954), the wild-type is denoted syn$^+$.

At least four loci which confer the syn phenotype to HSV-1 infected cells have been mapped (fig. 22) (Ruyechan et al., 1979; Little and Schaffer, 1981; DeLuca et al., 1982; Bond and Person, 1984; Kousoulas et al., 1984; Pogue-Geile et al., 1984). Three of the syn loci were mapped by Ruyechan et al. (1979) using HSV-1 x HSV-1 intratypic recombinants. These loci were designated syn1, syn2 and syn3.

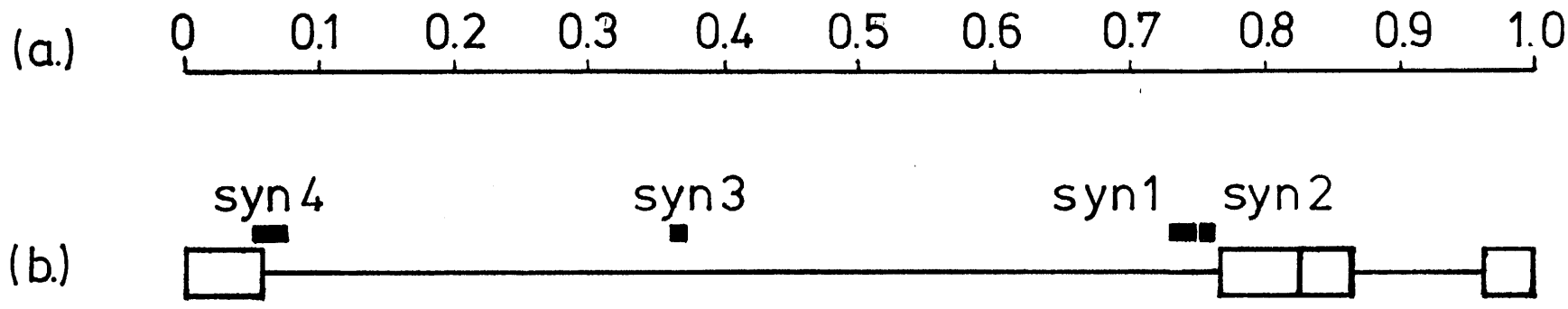

(a.)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
(b.)
syn4
syn3
syn1
syn2

FIGURE 22

Location of the mutations conferring the syn phenotype to HSV-1-infected cells. (a) is the fractional length (mu) of the HSV-1 genome in the prototype orientation (b). The open boxes represent the regions of the HSV-1 genome which contains the syn mutations. Ruyechan et al. (1979) originally mapped and designated the syn 1, 2 and 3 loci. DNA sequencing has recently located the syn 1 and 2 mutations (Debroy et al., 1985). The syn locus lying between co-ordinates 0.040 and 0.064mu was mapped by Little and Schaffer (1981) has been termed syn 4 only for the purpose of identification on this figure.

Ruyechan et al. (1979) presented evidence that the syn phenotype of HSV-1 (MP) results from expression of two separable mutations which lie between co-ordinates 0.68 and 0.82mu. The authors generated recombinants by transfer of DNA fragments from the syn phenotype strains HSV-1 (MP) or HSV-1 (O3) to a syn+ strain (F). Two classes of recombinant virus were obtained. Members of one class produced syn plaques on Vero cells, but syn+ plaques on HEp-2 cells. The locus responsible for the phenotype was designated syn1. Members of the other class produced syn plaques on both Vero cells and HEp-2 cells. The locus which, when present alone or in combination with syn1 is reponsible for the second phenotype, was designated syn2.

Recently, the mutation defining the syn1 locus (MP) and another mutant, syn20, giving the same phenotype was more precisely mapped to lie between co-ordinates 0.735 and 0.740mu (Pogue-Geile et al., 1984; Bond and Person, 1984). This region has now been completely sequenced and contains an open reading frame which potentially can encode a protein of 338 amino acids and has the characteristics of a transmembrane protein (Debroy et al., 1985). The nucleotide sequence of this same region of the syn-inducing mutants MP and syn20 was also determined. Both were shown to have amino acid substitutions at position 40 of the putative fusion protein. For mutant MP, the change was from alanine to valine while for syn20, it was alanine to threonine. A second alteration found only in MP (threonine to methionine at position 101) was thought not to affect fusion since it did not occur in syn20 and methionine was found at this residue in a syn+ strain of HG52.

A transcript from the putative fusion gene was detected and shown to be a late RNA in that it was not detected in the absence of DNA synthesis (Debroy et al., 1985).

Pogue-Geile et al. (1984) presented evidence to suggest that the

second syn locus of HSV-1 (MP), syn2, is probably not by itself a syn locus. They reasoned as follows: First, the syn2 locus cannot lie between 0.702 and 0.752mu since a fragment of MP DNA spanning these co-ordinates and containing the syn1 locus (0.735-0.740mu) produces large syn plaques on Vero cells but only small syn plaques on HEp-2 cells, the syn-1 phenotype in their hands. (The authors state that the reason for their difference in plaque morphology is not known). Second, marker rescue of MP with a fragment of MP DNA spanning co-ordinates 0.728-0.744mu, which from the first point above cannot contain the syn2 locus, permitted isolation of recombinants that displayed the syn+ phenotype on both Vero cells and HEp-2 cells. The syn2 locus remains therefore to be precisely mapped.

Syn3 was shown by Ruyechan et al. (1979) to map in the region of the gB-1 gene of the HSV-1 mutant tsB5. The syn mutation and the ts mutation of tsB5 are separable by recombination (Honess et al., 1980). More recently, the syn3 mutation has been shown by marker rescue experiments (DeLuca et al., 1982) and by DNA sequencing (Bzik et al., 1984b) to lie within the gB-1 structural gene. It therefore seems certain that gB-1 plays a role in cell fusion. The mutation at the syn3 locus was expressed in some cell types, but not in others (cell-type dependent) (Ruyechan et al., 1979; DeLuca et al., 1982).

The fourth syn locus was identified by Little and Schaffer (1981). Marker transfer of DNA fragments from the syn mutant to the syn+ parental HSV-1 strain KOS showed that this locus mapped between co-ordinates 0.040 and 0.064mu on the HSV-1 genome. Furthermore, the authors showed that expression of the mutation resulted in syn plaques on Vero, human embryo lung (HEL) and rabbit kidney (RK) cells, but syn+ plaques on HEp-2 cells, i.e. a phenotype like that of syn1. The mutant HSV-1 (KOS) 804 carrying this locus, failed to complement HSV-1 (MP) to

produce syn+ plaques (Little and Schaffer, 1981). Failure of complementation may be due to either, (i) both mutants being defective in an, as yet, unidentified locus affecting syn phenotypic expression, or (ii) HSV-1 (KOS) 804 carries, in addition to the syn4 mutation, the syn1 mutation which results in cell-type syn phenotypic expression, or (iii) HSV-1 (MP) carries, in addition to the syn1 and 2 mutations the syn4 mutation. However, examination of fig. 3 from Little and Schaffer (1981) shows that some transfer of syn phenotype was achieved with DNA fragments from HSV-1 (KOS) 804 which spans the syn1 locus. Furthermore, all marker rescue experiments were performed with DNA fragments excised from gels and the possibility of contaminating DNA fragments cannot be excluded. What is needed to clarify the situation is for the experiments to be repeated with the putative syn4 mutation cloned into a wild-type genome.

A syn mutant, HSV-1 (KOS) 78R, was isolated and characterised by Little and Schaffer (1981). It was shown to be temperature sensitive for the syn phenotype. The locus was mapped by marker rescue to be between co-ordinates 0.724 and 0.747mu and the mutant expressed the syn phenotype in Vero and HEL cells, but not in RK cells at the NPT. This is comparable with the syn1 locus of HSV-1 (MP). However, HSV-1 (MP) and HSV-1 (KOS) 78R complemented to yield syn+ plaques. This suggests that the locus responsible for the syn phenotype of HSV-1 (KOS) 78R lies in a different complementation group from that of syn1 of HSV-1 (MP) and therefore may be a fifth syn locus.

Deletions in the TK gene resulted in syn phenotype expression. However, it is not known whether one of these deletions or another, as yet, undetected mutation, resulted in the syn phenotype (Sanders et al., 1982).

It has been established that the syn mutant HSV-1 (MP) (Hoggan and Roizman, 1959) is also gC negative (gC-) (Heine et al., 1974;

Manservigi et al., 1977). Analysis of recombinants between HSV-1 (MP) and HSV-1 (tsB5) led Manservigi et al. (1977) to propose that gB-1 is involved in cell fusion and that expression of gC-1 can suppress this activity. Other syn mutants are also gC$^-$ (Cassai et al., 1975, 1976; Zezulak and Spear, 1984b) suggesting a relationship between absence of expression of gC and cell fusion. While some as yet undefined relationship between the two phenotypes appears to hold, it is not a simple one. Examples exist of virus which fail to express gC and have syn$^+$ morphology (Honess and Watson, 1977; Honess et al., 1980) as do examples of virus which express gC and form syncitia in Vero cells (Pogue-Geile et al., 1984). Moreover, insertion of a functional gC-1 gene into the TK gene of HSV-1 (MP) resulted in expression of gC-1 without changing the syn phenotype (Lee et al., 1982b). Further work is needed to understand what role, if any, gC plays in expression of the syn phenotype.

Evidence presented by Noble et al. (1983) indicates that gD-1 plays a role in HSV-1-induced cell fusion. The authors showed that fusion of Vero cells infected with the syn strain HSV-1 (HFEM) syn was inhibited by addition of monoclonal antibodies directed against gD. Surprisingly no such inhibition was observed when anti-gB monoclonal antibodies were added to the infected cells. The authors showed by gluteraldehyde fixation that the anti-gB monoclonal antibodies did bind to the infected cells, suggesting that either the antibodies tested do not bind to the appropriate determinant critical for cell fusion or that the role of gB-1 is indirect.

The mechanism of cell fusion is unknown, but presumably proteins integrated in the membrane of adjacent infected cells react and thus promote fusion. Examples of viral-coded glycoproteins which are known to form oligomers are gB-1 (Sarmiento and Spear, 1979; Haarr and Marsden, 1981; Palfreyman et al., 1983) and gD-1 (Eisenberg et al., 1982b; Palfreyman et al., 1983; this thesis) and are therefore candidates for

being involved in this putative cell fusion mechanism.

1.22.5 Expression of receptors on the cell surface membrane

(a) Fc receptors

Watkins (1964) reported that antibody-sensitised erythrocytes, but not untreated erythrocytes, would bind to HSV-1-infected HeLa cells. It was later shown that other cell lines infected with HSV-1 or HSV-2 could bind to antibody, specifically immunoglobulin G (IgG), and that binding was to the Fc region of IgG (fig. 23) (Yasuda and Milgrom, 1968; Westmoreland and Watkins, 1974; Feorino et al., 1977; Costa et al., 1978; McTaggart et al., 1978; Nakamura et al., 1978; Bourkes and Menezes, 1979; Cines et al., 1982). Similar Fc-receptors are induced by other herpesviruses, these are human cytomegalovirus (HCMV) (Keller et al., 1976; Rahman et al., 1976; Westmoreland, 1976) and varicella zoster virus (VZV) (Ogata and Shigeta, 1979). Johansson et al. (1984) have examined the specificity of the HSV-1-induced Fc-receptor. IgG4 binds more strongly than IgG1 or IgG2. IgG3 does not bind to the Fc-receptor, nor do the other immunoglobulins. Para et al. (1980) showed that the HSV-1-induced Fc-receptors are on the surfaces of virions as well as infected cells. Using the technique of ^{125}I surface labelling and electron microscopy, they showed that expression of Fc-binding receptors on the surface of infected cells occurs immediately after exposure of cells to purified virions and in the absence of viral gene expression. Thus, the Fc-receptors are probably a virion component acquired by the cell upon fusion of the virion envelope with the cell membrane.

Baucke and Spear (1979) identified, by Fc-affinity chromatography, an HSV-1-induced glycoprotein which had affinity for the Fc region of IgG which they designated gE. Later Para et al. (1982a) showed that $F(ab)_2$ fragments (see fig. 23) from anti-gE-1 serum could partially block Fc

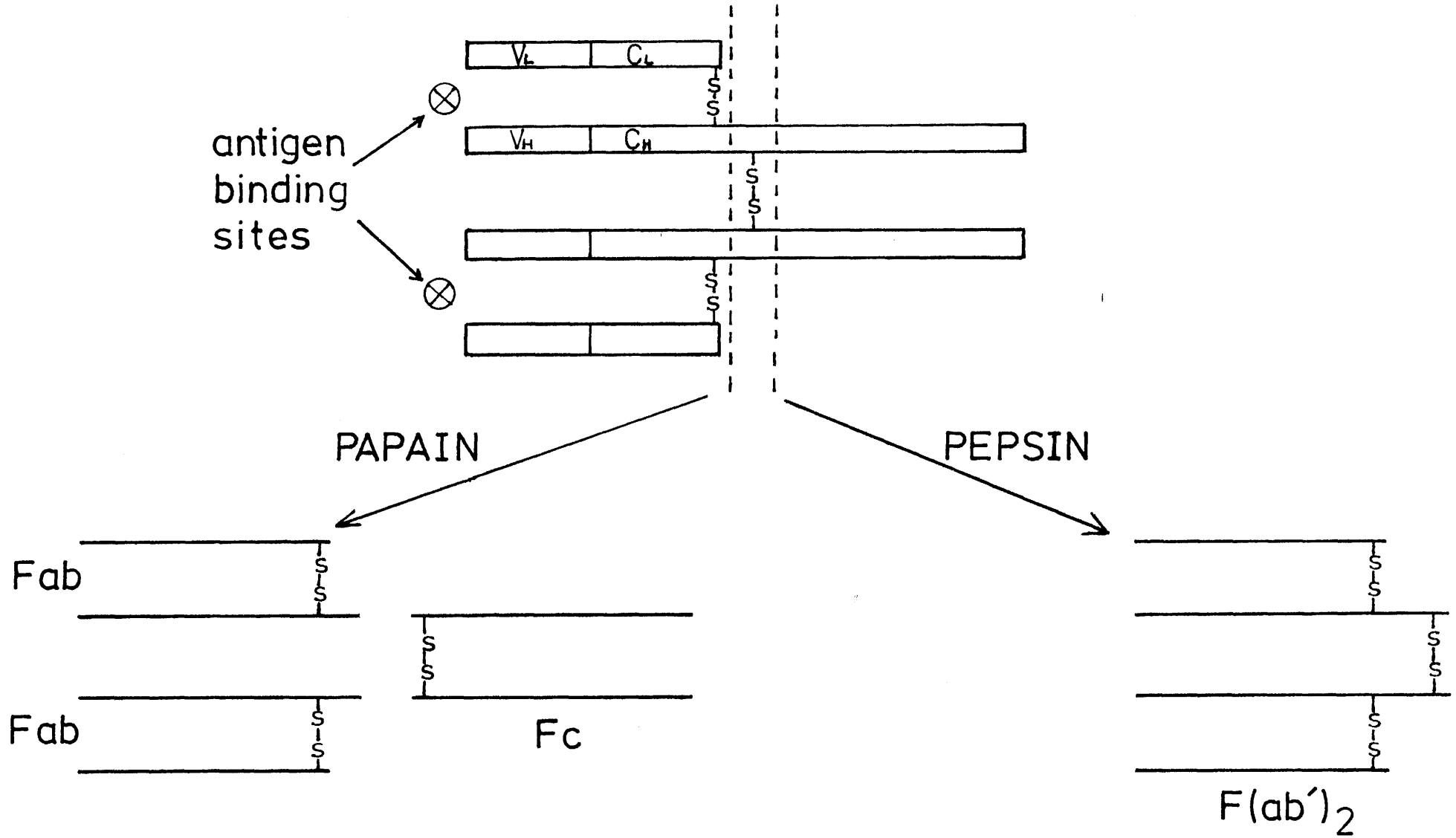

antigen
binding
sites
V_L
C_L
V_H
C_H
PAPAIN
PEPSIN
Fab
Fab
Fc
$F(ab')_2$

FIGURE 23

Representative structure of an antibody or immunoglobulin (Ig), the figure is taken from Roitt (1984). Top, Ig consists of two heavy and two light polypeptide chains held together by disulphide bonds. The terms V_L and C_L designate the variable and constant regions, respectively, of the light chain. Similarly V_H and C_H designate the variable and constant regions of the heavy chains.

binding activity. These data, together with the observation that gE is present on the surface of infected cells and virions, strongly suggest that gE is the HSV-induced Fc-receptor.

The physiological significance of the HSV-induced Fc-receptor is not understood. Hypotheses concerning its functional role have been proposed by several investigators (Costa and Rabson, 1975; Lehner et al., 1975; Adler et al., 1978; Costa et al., 1977). Costa et al. (1977) showed a 99% reduction in virus production when HSV-2-infected Vero cells were incubated in the presence of 10mg ml^{-1} of IgG from normal rabbit serum, compared to a control culture in which the IgG was replaced with albumin. The authors hypothesised that such physiological concentrations of IgG may be of relevance in control of primary infection and in the establishment or maintenance of latency. However, this result does not appear to have been followed up and it is of some concern that no estimate of the purity of the IgG was given and that no inhibition of virus growth was observed when the concentration of IgG was reduced ten-fold to 1mg ml^{-1}.

The HSV-induced Fc-receptor may be involved in mechanisms of escape from immune cytolysis (Adler et al., 1978). In this instance, it may be relevant that the binding affinity of IgG subclasses to the Fc-receptor (IgG>IgG2>IgG1, IgG3 is not bound) is the inverse to the binding affinity of the C1q component of complement (involved in antibody-dependent, complement mediated cytolysis), which is IgG3>IgG1>IgG2>IgG4 (Schumaker et al., 1976).

(b) C3b receptor

Dierich et al. (1979) showed that primary RK cells infected with HSV-1, but not uninfected cells, bound Raji lymphoblastoid cells to which purified C3 component of complement was attached. Furthermore, the presence of protease inhibitors suppressed the interaction of the infected

cells with the C3 bound Raji cells. The authors interpreted their results as indicating de novo expression of protease activity on the surface of HSV-1 infected cells. These experiments are particularly intriguing when viewed in the light of more recent experiments of Cines et al. (1982) who showed that human endothelial cells infected with HSV-1 develop receptors for the C3b component (the activated form of C3) of complement.

Friedman et al. (1984) demonstrated that gC-1, but not gC-2, functions either alone or in combination with endothelial cell proteins, as a receptor for the C3b component. The C3b receptors were detected using erythrocytes sensitised with IgM and purified complement components. The C3b receptors could not be detected when anti-gC-1 monoclonal antibodies were added to the infected cells before adding the sensitised erythrocytes. In addition, HSV-1 (MP) which does not synthesise gC, does not express a C3b receptor. Thus, these results show that gC-1 forms part of, or is, the C3b receptor. More recently, both gC-1 and C3b have been purified to homogeneity (as judged by silver staining gels of the purified proteins) and a direct interaction between them has been demonstrated (Cohen et al., Tenth International Herpesvirus Workshop, Ann Arbor, 1985).

Friedman et al. (1984) observed that gC-2 does not function as a C3b receptor. Comparison of the DNA sequence data for the genes encoding gC-1 (Frink et al., 1983) and gC-2 (Dowbenko and Lasky, 1984; Swain et al., 1985) show that there is a sequence of twenty-eight amino acids in gC-1 which is not present in gC-2. It is possible that there are sequences within the twenty-eight amino acids responsible for the binding of C3b.

Activation of the complement pathway can lead to the lysis of infected cells (for review, see Roitt, 1984). Generation of the C3b component can lead to a positive feedback mechanism in which the product of C3 breakdown (i.e. C3b) combines with other proteins to form an

enzyme which cleaves C3 to generate more C3b. Fearon (1979) presented evidence that C3b receptors on erythrocytes can inhibit the activation of the complement pathway, possibly by impairing the function of bound C3b, and consequently preventing generation of the enzyme which cleaves C3. If gC-1 inhibits complement activation in this way, its presence on the surface of infected cells may reduce the efficiency of lysis of infected cells by the host. Consequently, mutants which do not express gC-1 might be expected to be less pathogenic. This prediction has now been experimentally observed (Sunstrum et al., Tenth International Herpesvirus Workshop, Ann Arbor, 1985).

1.22.6 The egress of virions from infected cells

The HSV virion acquires its envelope primarily at the inner nuclear membrane (Falke et al., 1959; Morgan et al., 1959). It then must be transported from the perinuclear space to the outside of the cell. Morgan et al. (1959) proposed, on the basis of EM studies, egress of virions occurred by a process of reverse phagocytosis involving transport vesicles operating between the rough endoplasmic reticulum and the Golgi complex and between the Golgi complex and the cell surface. Schwartz and Roizman (1969) proposed, on the basis of EM studies, that egress of virions is via a network of tubules observed in infected cells. As yet, there is no evidence that any particular glycoproteins are essential for the egress of virions.

Some syn strains of HSV do not synthesise gC (Heine et al., 1974; Manservigi et al., 1977; Cassai et al., 1975, 1976; Zezulak and Spear, 1984b). This suggests that gC is not necessary for the egress of virions.

More recently, Buckmaster et al. (1984) observed that a monoclonal antibody directed against gH-1 inhibits plaque formation, whereas anti-gD monoclonals do not. The authors speculated that inhibition of plaque

TABLE 9

Randall et al. (1980)		This Thesis	Norrild and Vestergaard (1979)	Chen et al. (1978)	
Nomenclature	Apparent MW ($x10^{-3}$) by SDS-PAGE	Apparent MW ($x10^{-3}$) by SDS-PAGE	Nomenclature	Nomenclature	Apparent MW ($x10^{-3}$) by SDS-PAGE
HSV-1					
ICRP a	124K - 138K	112K - 120K	Ag6, Ag11	A1	132K
ICRP b	59K - 65K	57K			
ICRP c	50K - 54K	45K - 47K	Ag8		
ICRP d	43K - 45K	40K			
ICRP e	30K - 35K	32K 34K 35K			
ICRP f	21K - 22K				
HSV-2					
ICRP 1	86K - 100K	92K			
ICRP 2	70K - 76K	85K and 74K			
ICRP 3	66K - 69K	63K			
ICRP 4	52K - 56K	50K	Ag8		
ICRP 5	ND	40K			
ICRP 6	ND				

TABLE 9

Comparison of the nomenclatures used to describe proteins secreted from HSV-infected cells. In the table are listed the proteins secreted from HSV-1- and HSV-2-infected cells which were identified in this study and the studies of Randall et al. (1980), Norrild and Vestergaard (1979) and Chen et al. (1978). Correlations of nomenclature were based on MW determinations and/or immunologic data (Randall et al., 1980; Norrild et al., 1979; Norrild, 1980). ICRP = infected cell released polypeptide; ND = no data available for the MW of these proteins; a blank space indicates that a correlation cannot be made because either insufficient evidence is available to allow comparison or no other secreted proteins have been identified by the authors. Other secreted proteins are mentioned in the text, but not in this table, are Ag3, Ag4A, Ag7 and Ag9 (Norrild and Vestergaard, 1979) and peak C (Chen et al., 1978) cannot be assigned identity to any other known secreted protein.

comparison of immunoprecipitations with antisera prepared from rabbits inoculated with purified individual glycoproteins, it was established that Ag6, Ag8 and Ag11 correspond to gC, gD and gB respectively (Norrild et al., 1979; Norrild, 1980). The identities of Ag3, Ag3A, Ag4, Ag4A and Ag9 are unknown. However, an HSV-2 glycoprotein, g92K (Marsden et al., 1978, 1984; this thesis) is a possible candidate for identity with Ag4, Ag4A or Ag9.

Chen et al. (1978) compared virion and secreted HSV-1 glycoproteins. Of the three identified virion glycoproteins, designated AI, AII, and B, only AI was secreted together with a low MW protein, designated C, not found in virions. Based on the apparent MWs and the gel system used, it is likely that glycoproteins AI, AII and B, correspond to gC, gB and gD respectively. A comparison, based on MWs and/or immunological data, of the nomenclatures used by Chen et al. (1978), Randall et al. (1980) and Norrild and Vestergaard (1979) is shown in Table 9.

The data of Chen et al. (1978) appears inconsistent with that of Randall et al. (1980), Norrild and Vestergaard (1979) and this thesis, in that, Chen et al. (1978) identifies only two secreted glycoproteins (AI (gC) and C). The inconsistency may be a consequence of the method used by Chen et al. (1978) to radiolabel the glycoproteins. Infected cells were maintained and radiolabelled in medium devoid of serum. Data presented in this thesis (see Section 3.8.7) shows that a fragment of gE-1 is secreted only if serum is present in the medium. The inconsistency could conceivably be caused by the strain of virus used.

MATERIALS AND METHODS

CHAPTER 2

MATERIALS AND METHODS

MATERIALS

Cells

Baby hamster kidney (BHK) 21 clone 13 cells (Macpherson and Stoker, 1962) and human foetal lung (HFL) cells established by Dr. B. Carritt were used for these experiments.

Virus

Herpes simplex virus type 1 strain 17 syn+ (Brown et al., 1973) and herpes simplex virus type 2 strain HG52 (Timbury, 1971) were the wild type viruses used throughout this study.

The ts mutant of strain 17 syn+, tsK, has been described previously (Marsden et al., 1976), as have recombinants 17+x11r (1), Bx6 (17-1), RE6, RH6, RS5 (Marsden et al., 1978), RE4 (Wilkie et al., 1979), Bx1 (28-1-1) (Davison et al., 1981), RD103, RD104, RD113, RD119, RD213, RD216 (Davison, 1981; Marsden et al., 1982) and R12-1 (Chartrand et al., 1981).

Tissue culture media

BHK cells were cultured using Glasgow modified Eagle's medium (Busby et al., 1964), supplemented with 50 ug/ml gentamicin (Flow Laboratories, Irvine, Scotland) and 0.002% phenol red. Calf serum was added to give a final concentration of 10% (EC10). HFL cells were also cultured in Glasgow modified Eagle's medium, but supplemented in addition to the above with 1% non-essential amino acids (NEAA) and 1% sodium pyruvate (Flow Laboratories, Irvine, Scotland). Foetal calf serum was added to a final concentration of 10% (EFC10).

During experiments variations of the basic media described above were used:-

EC2	Eagle's medium containing 2% calf serum;
EFC2	Eagle's medium containing 2% foetal calf serum;
E(-met)	Eagle's medium lacking methionine;
$\frac{\text{Emet}}{5}$C2	Eagle's medium containing one-fifth the normal concentration of methionine and 2% calf serum;
$\frac{\text{Emet}}{5}$FC2	Eagle's medium containing one-fifth the normal concentration of methionine and 2% foetal calf serum;
E(SO4free)	Eagle's medium in which $MgCl_2$ was substituted for $MgSO_4$;
E(SO_4free)C2	Eagle's medium, in which $MgCl_2$ was substituted for $MgSO_4$, and containing 2% calf serum;
E(SO_4free)FC2	Eagle's medium in which $MgCl_2$ was substituted for $MgSO_4$ and containing 2% foetal calf serum;
EHu10	Eagle's medium containing 10% human serum;
EHu5	Eagle's medium containing 5% human serum;
EHu2	Eagle's medium containing 2% human serum;

STANDARD SOLUTIONS

Phosphate buffered saline (PBS)

170mM NACl, 3.4mM KCl, 1mM Na_2HPO_4, 2mM KH_2PO_4, pH7.2 in distilled water (Dulbecco and Vogt, 1954).

Tris-Saline

140mM NaCl, 30mM KCl, 0.28mM Na_2HPO_4, 1mg/ml dextrose, 0.25mM Tris, 0.005% phenol red (pH7.4) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin.

Versene

EDTA (6mM) dissolved in PBS containing 0.0015% phenol red.

Trypsin-Versene

One volume of 25% (w/v) Difco trypsin (dissolved in Tris-saline) plus four volumes of versene.

Giemsa stain

1.5% (v/v) suspension of Giemsa in glycerol, heated at 56°C for 1½-2h and diluted with an equal volume of methanol.

Enzymes and metabolic inhibitors

Tunicamycin was obtained from BDH. Deoxyribonuclease I (DN 100) and ribonuclease A (R-4875) were obtained from Sigma. L-1-tosylamide-2-phenylethylchloromethyl ketone (TPCK)-treated trypsin (TRTPCK 37H 895P) was purchased from Worthington Biochemicals Corporation, New Jersey.

Monoclonal antibodies

The monoclonal antibodies directed against gD (2001/1) and gB (2975) were isolated by Dr. J.W. Palfreyman by the method described in Palfreyman et al. (1983). The monoclonal antibodies directed against gE (3104) and the secreted proteins (3114/109) were isolated by Dr. A. Cross. Their isolation and characterisation will be described elsewhere (A. Cross, R.G. Hope and H.S. Marsden, manuscript in preparation). The monoclonal antibodies, AP1 and LP5, were isolated by Dr. A.C. Minson and identified by immunoprecipitation as described by McLean et al. (1982). The rabbit monospecific antiserum to gE-1 was a gift from Dr. P.G. Spear and its production was described by Para et al. (1982a).

Radiochemicals

All radiolabelled compounds used in this study were obtained from Amersham International plc:-

(i) ^{35}S-labelled inorganic sulphate (specific activity 25-40 Ci/mg);

(ii) [^{35}S]-L-methionine (specific activity >600 Ci/mmol.);

(iii) D[1-^{14}-C] glucosamine hydrochloride (specific activity 50-60 mCi/mmol.);

(iv) D-[2-^{3}H] mannose (specific activity 10-20 Ci/mmol.).

Electrophoresis materials

Acrylamide used for one-dimensional slab gel electrophoresis was supplied by Koch-Light Laboratories, Haverhill, Suffolk, England and N,N',-methylenebisacrylamide (bis) was supplied by BDH Chemicals Ltd., Poole, Dorset, England. The acrylamide and bis used for non-equilibrium pH gradient gel electrophoresis (NEPHGE) in two-dimensional electrophoresis were from Biorad Laboratories, Richmond, California. The TEMED (N, N, N', N'-tetramethylethylenediamine) and ammonium persulphate were also supplied by Biorad Laboratories. Urea (ultra pure grade) was from Bethesda Research Laboratories, Cambridge, England. Ampholines were from LKB, Bromma, Sweden. All other chemicals were from either Sigma (London) Chemical Company, Kingston-upon-Thames, London or from BDH and were of analytical reagent grade or better.

Immunoprecipitation materials

Heat-killed and formalin-fixed Staphylococcus aureus was obtained from Bethesda Research Laboratories, Cambridge, England. Rabbit anti-mouse ascites fluid was supplied by Miles-Yeda Ltd., Kiryat Weizmann, Rehovot, Israel.

Immunoaffinity and Fc-affinity chromatography

Cyanogen bromide-activated Sepharose 4B was supplied by Pharmacia Laboratories, Upsalla, Sweden. For Fc-affinity chromatography, bovine serum albumin (fraction v) was purchased from Sigma Laboratories and anti-bovine serum albumin (rabbit) was from Miles-Yeda Laboratories.

Tryptic peptide mapping materials

Formic acid (AR), butan-1-ol (AR), hydrogen peroxide and pyridine (spectroscopy grade) were purchased from BDH. Glacial acetic acid was purchased from Koch-Light. Eastman chromatogram sheets (13255) were purchased from Eastman Kodak, Rochester, New York; PD10 columns (prepacked Sephadex G25 columns were purchased from Pharmacia Laboratories.

Autoradiography and fluorography

Agfa Scopix CR3 NIF or Kodak X-Omat XS1 film was used for autoradiography or fluorography respectively. En^{3}Hance from New England Nuclear was used for fluorography.

Miscellaneous materials

Teflon tape used for sealing gel sandwiches was purchased from Minnesota Mining and Manufacturing Company. Plastic petri dishes were from Flow Laboratories, Irvine, Scotland.

METHODS

2.1 Growth of cells

BHK cells were grown at 37°C in rotating 80oz bottles containing 200ml EC10 in an atmosphere of 5% CO_2 and 95% air. HFL cells were grown in 800ml plastic tissue culture flasks containing 50ml EFC10 under the same atmosphere. Confluent cells were harvested by washing the monolayers twice with trypsin/versene and the detached cells were resuspended in about 20ml of fresh medium. BHK cells were stored for up to five days at 4°C but HFL cells were replated immediately. For experiments, cells were seeded onto 50mm or 30mm plastic petri dishes.

Serum starved cells were prepared by the method of Howard et al. (1974). Such cells exhibit 1% of the rate of DNA synthesis of non-starved cells (Burk, 1967).

2.2 Growth of virus stocks

Almost confluent BHK cells in 80oz roller bottles were infected at a multiplicity of infection (moi) of one plaque forming unit (pfu) per 300 cells in 40ml EC10 at 31°C. When maximum cytopathic effect (cpe) was obtained (about four days), virus was harvested by the following method. Cells were shaken off into the medium and centrifuged at 1500xg for 10min. The pellet was resuspended in 5ml of supernatant, checked for sterility and stored at -70°C. The supernatant from the 1500xg spin was centrifuged at 25000xg for 2h and the resulting pellet resuspended in 5ml of its supernatant. This suspension was also checked for sterility (as described in Section 2.4) and stored at -70°C. Sterile suspensions of the 25000xg pellet were treated in a bath sonicator (50W at +4°C for approximately 1min.) and termed the supernatant virus stock. Sterile suspensions of the 1500xg pellet was similarly sonicated for 4x1min in the bath sonicator then clarified by centrifugation at 1500xg for 10min. The

resulting supernatant was decanted and stored (supernatant 1). The pellet was resuspended in 5ml EC10, re-sonicated as above then centrifuged at 1500xg for 10min. The resulting supernatant was added to supernatant 1 and termed the cell-associated stock.

Both supernatants and cell-associated stocks were titrated at 31°C and 38.5°C (section 2.3) and stored in 0.5ml aliquots at -70°C.

2.3 Titration of virus stocks

Stocks to be titrated were serially diluted from 10^{-1} to 10^{-6} in PBS+ Ca5%. Aliquots of 0.2ml were added to almost confluent BHK cell monolayers on 50mm plastic petri dishes, in duplicate. After 1h adsorption, the inoculum was removed, 4ml of EHu5 was added and the cells incubated for 2 days at 37°C or 38.5°C or for 3 days at 31°C. Monolayers were fixed with Giemsa stain (10min. at room temperature) and plaques were counted using a dissection microscope.

2.4 Sterility checks on HSV stocks

Virus stocks were tested for sterility on both brain heart infusion agar (BHI) and brain heart infusion agar plus 10% horse blood (BHI blood agar).

2.5 Preparation of radio-labelled infected-cell polypeptides and secreted protein samples

Confluent monolayers in 30mm petri dishes were infected with approximately 20 pfu/cell. After 1h incubation at the appropriate temperature, virus was removed and the infected cell monolayer was washed twice with labelling medium (see below). Incubation was continued in 1ml of that medium. Infected-cell monolayers were radio-labelled as follows:-

(a) Long label

(i) [^{35}S]-methionine (specific activity >600 Ci/mmol.) at a concentration of 10 uCi/ml in $\frac{EmetC_2}{5}$;

(ii) [^{35}S]-inorganic sulphate (specific activity 25-40 Ci/mg) at a concentration of 500 uCi/ml in E(SO_4 free)C_2;

(iii) D-[1-^{14}C] glucosamine hydrochloride (specific activity 50-60 mCi/mmol) at a concentration of 10 uCi/ml in EC2.

Radioactive label was added 2h after the end of virus adsorption and cultures were harvested about 20h later. Where indicated, the drug tunicamycin was added at a final concentration of 2 ug/ml. The drug was added to monolayers about 20min. before infection and maintained there at all times until they were harvested. Mock-infected cultures were treated in a similar manner, except that virus was omitted. The secreted protein samples were prepared by centrifuging the growth medium from the monolayers at 35000xg for 2h to remove virions.

(b) Pulse-label

To pulse-label cells, the growth medium (Eagle's medium, with or without sulphate as appropriate, and supplemented with 2% calf serum) was removed at 5h post-infection and the radioactive label added in PBS supplemented with 1% non-essential amino acids. The radioactive label was either ^{35}S-inorganic sulphate at a concentration of 500 uCi/ml or D[2-^{3}H] mannose (specific activity 10-20 Ci/mmol.) at a concentration of 100 uCi/ml (the ethanol in which mannose was supplied was removed before use). Where indicated, tunicamycin was added as described above. Control mock-infected cultures were treated in a similar manner except that virus was omitted. The secreted protein samples were prepared as

described above.

(c) Pulse-chase experiments

Cells were labelled as described for the pulse-label above, with ^{35}S-inorganic sulphate at a concentration of 500 uCi/ml for the times indicated in the figure legends and either harvested immediately (pulse) or chased for various times by removing the label, washing the monolayers three times with EC2 and then incubating at 37°C until harvesting. The secreted protein samples were prepared as described above.

2.6 Preparation of immunoaffinity columns

Monoclonal antibodies were first purified from ascites fluid as described below then linked to cyanogen bromide-activated sepharose 4B (Pharmacia) using standard methods as described by the manufacturer and outlined below.

2.6.1 Purification of monoclonal antibodies

About 2ml mouse ascites fluid containing monoclonal antibody was centrifuged at 50000xg for 30min to remove particulate matter. The centrifuged ascites fluid was diluted with three volumes of PBS. An equal volume of saturated ammonium sulphate was then added and left to stand at +4°C for 1hr to precipitate protein which was pelleted by centrifugation at 1500xg for 10min. Pellets were resuspended in 1ml of PBS then dialysed against two changes of 2 litres bicarbonate buffer (0.1M $NaHCO_3$, 0.5M NaCl, pH8.3) at +4°C.

The dialysed ascites fluid was then fractionated on a Sephacryl S200 column (1cm x 16cm). Forty fractions each of 1.5ml were collected using bicarbonate buffer and the optical density of each fraction

determined on a CE595 double beam digital UV spectrophotometer (Cecil Instruments). Aliquots of each fraction were denatured then analysed by SDS-polyacrylamide gel electrophoresis (Section 2.9). Peak fractions of IgG were pooled and used to prepare immunoaffinity chromatography columns (see Section 2.6.2).

2.6.2 Linkage of purified monoclonal antibody to sepharose 4B

1g of dried cyanogen bromide-activated sepharose 4B was swelled for 15min. by addition of approximately 10ml of 1mM HCl. The beads were washed with approximately 200ml of 1mM HCl, followed by two washes of 20ml bicarbonate buffer. To the swelled and activated beads was added the purified immunoglobulin (5mg IgG/1ml of swelled beads). The IgG was covalently linked to the beads by mixing at room temperature for 2h in an end over end mixer. After the two hours, excess active sites on the beads were blocked by adding 1M ethanolamine, pH8.0 and mixing for 2h at room temperature in an end over end mixer. The immunoadsorbent was washed two times with alternate washes of bicarbonate buffer, pH8.3, then acetate buffer pH4.0 (0.1M Na^+ CH_3COO^-, 0.5M NaCl). The coupled IgG (about 3.5ml/1g dried sepharose) was poured into a column 1cm wide to form the immunoadsorbent column.

2.7 Preparation of Fc-affinity columns

Bovine serum albumin (BSA) was covalently linked to cyanogen bromide-activated sepharose 4B according to the procedure of Pharmacia above (Section 2.6.2). Fc-affinity columns were prepared as described by Baucke and Spear (1979). Briefly, serum containing specific anti-BSA IgG was passed slowly through the BSA-sepharose column for about 1h at room temperature. Unbound protein was washed out with excess PBS + 0.5% NP40. The columns were equilibrated before use (see Section 2.8.2).

2.8 Affinity chromatography

2.8.1 Immunoaffinity chromatography

The extracts from which the proteins were to be purified by immunoaffinity chromatography were prepared as follows: Infected-cell monolayers were prepared as described in Section 2.5, then suspended in 0.5ml each of 0.01M Tris, 0.15M NaCl, 0.5% NP40, 0.5% sodium deoxycholate, pH7.5, incubated at +4°C for 1h, sonicated (see Section 2.2) then centrifuged at 35000xg for 2h.

The extracts were loaded onto the column and allowed to incubate with the immunoadsorbent for about 2h at +4°C (each sample contained about $5x10^6$ cell equivalents). Unbound protein was then washed out of the column with excess buffer (0.01M Tris, 0.5M NaCl, 0.1% NP40, pH7.5). Bound protein was eluted with 50ml of 3M NaSCN, 0.01M Tris, pH7.8. Eluted protein was concentrated to 1ml in a dialysis sac surrounded by polyethyleneglycol (PEG) 6000 then dialysed against 2 litres PBS overnight at +4°C. Samples were denatured and analysed by SDS-PAGE (Section 2.10).

2.8.2 Fc-affinity chromatography

This was performed essentially as described by Baucke and Spear (1979). Monolayers were solubilised in PBS + 0.5% NP40 and kept on ice with occasional mixing for 15min. Nuclei were then removed by centrifuging at 1500xg for 10min.

The extract containing the infected cell membranes (supernatant) was added to the Fc-affinity columns at 37°C for about 15min. after which the columns were washed extensively with PBS + 0.5% NP40. Column bound protein was eluted with 3M NaSCN, 0.01M Tris, pH7.8, in PBS + 0.5% NP40 then concentrated with PEG 6000 as described in Section

2.8.1. Samples were then denatured and analysed by SDS-PAGE (Section 2.10).

2.9 Immunoprecipitations

The immunoprecipitation methods described here and below are based on the binding of antigen-antibody complexes to heat-killed and formalin-fixed Staphylococcus aureus (Kessler, 1975).

2.9.1 Immunoprecipitation of gD

The method used to immunoprecipitate gD was described by Zweig et al. (1980). Infected or mock-infected cells were solubilised with 0.1M Tris, 0.5% NP40, 0.5% Na deoxycholate, 10% glycerol, pH8.0 ($5x10^6$ cells/1ml), sonicated (Section 2.2) for 1min., then left at +4°C for 1h. Particulate matter was removed by centrifugation at 13000 rpm in a microfuge for 5min. Extracts were clarified by centrifugation at 60000xg for 1h and the supernatant retained for immunoprecipitations.

Antibody (20ul of ascites fluid) was incubated with 0.5ml of cell extract ($\sim 2x10^6$ cell equivalents) or 0.5ml of supernatant extract for 3h at +4°C then further incubated for 1h with 100ul Staphylococcus aureus (Staph. aureus). The mixture was centrifuged in a microfuge at 13000 rpm for 1min. then the pellet was washed three times with 0.5M LiCl, 0.1M Tris, 1% beta-mercaptoethanol, pH8.0. The washed pellets were resuspended in 0.5ml. 2% sodium dodecyl sulphate (SDS), 20% glycerol, 2.5% beta-mercaptoethanol, 0.05M Tris, pH8.0, then boiled for 5min. and analysed by SDS-PAGE (Section 2.10).

2.9.2 Immunoprecipitation of gE

gE was immunoprecipitated as described by Lee et al. (1982a). Mock-infected or infected cells were solubilised in 140mM NaCl, 20mM

Tris, 1% NP40, 0.5% Na deoxycholate, 0.1% ovalbumin, pH7.6 (1ml/5x10^6 cells), sonicated for 1min. (Section 2.2) then centrifuged at 100000xg for 1h. 10ul of monospecific antiserum was incubated with 100ul of clarified antigen (the supernatant) for 1h at 4°C, after which time 100ul of Staph. aureus was added and incubated for another 1h. The mixture was then centrifuged at 13000 rpm in a microfuge for 1min. The pellet which contains Staph. aureus plus bound antigen-antibody complex was washed three times by centrifugation in a microfuge at 13000 rpm for 1min., first, with 140mM NaCl, 20mM Tris, 1% NP40, 0.5% Na deoxycholate, 0.1% SDS, pH7.6, second, with 140mM NaCl, 20mM Tris, 0.1% NP40, pH7.6, third, with 10mM Tris, 0.1% NP40, pH7.6 with 1min. The final pellet was resuspended in denaturing buffer (Section 2.10), boiled for 5min. then analysed by SDS-PAGE (Section 2.10).

2.9.3 Immunoprecipiation of g92K

Cells were solubilised in lysis buffer (0.9M NaCl, 0.01M Tris, 7.5mM-L-methionine, 10% triton-X-100, 10% Na deoxycholate, pH7.4) diluted tenfold in PBS, to a concentration of 5x10^6 cell equivalents/1ml then left at +4°C for 20min. Insoluble material was removed by centrifugation at 13000 rpm for 1min. in a microfuge. g92K was immunoprecipitated by the method of McLean et al. (1982). Antibody (5ul of ascites fluid) was incubated with radioactively labelled antigen for 30min. at room temperature before adding 25ul of Staph. aureus. This mixture was kept at room temperature for 2h. Staph. aureus (with antigen-antibody complexes attached) were centrifuged in a microfuge at 13000 rpm for 1min. then washed three times with 0.5M LiCl, 0.1M Tris, 1% beta-mercaptoethanol, pH7.4. The final pellet was resuspended in 0.1ml of denaturing buffer and boiled for 5min. then analysed by SDS-PAGE (Section 2.10).

2.10 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

2.10.1 One-dimensional (1-D) SDS-PAGE

Slab gels were cast vertically in a sandwich consisting of two glass plates separated by 1.5mm thick perspex spacers and sealed with teflon tape. Three types of resolving gel were used: (i) single concentration gels containing 10% acrylamide cross-linked with 1 part in 40 (w/w) N,N'-methylenebisacrylamide in 1.5M Tris, pH8.9, 0.4% SDS, (ii) single concentration gels containing 9% acrylamide cross-linked with 1 part in 40 (w/w) N,N'-diallyltartardiamide in 1.5M Tris, pH8.9, 0.4% SDS, (iii) gradient gels contained a 5% to 12.5% linear gradient of acrylamide cross-linked with 1 part in 20 (w/w) N,N'-methylenebisacrylamide also in 1.5M Tris, pH8.9, 0.4% SDS. Immediately after casting, the gel was overlayed with butan-2-ol in order to ensure a smooth interface on polymerisation. Polymerisation was achieved by the addition of ammonium persulphate (0.006%) and N,N,N',N', tetramethylethylenediamine (TEMED) (0.004%) to the gel solution before the gels were loaded.

Stacking gels were buffered with 0.49M Tris, pH6.7, containing 0.4% SDS and were polymerised just prior to sample loading. The gels contained 5% acrylamide cross-linked with the same chemical and at the same ratio as was used in the resolving gel.

Samples were prepared for analysis by boiling for 5min. in a denaturing buffer with a final concentration of 50mM Tris, pH6.7, 2% SDS, 5% beta-mercaptoethanol, 10% glycerol. Gels were electrophoresed at either 0.45mA/cm^2 for 3-4h or 0.09mA/cm^2 for 18h using a buffer containing 52mM Tris, 67mM glycine and 0.1% SDS.

2.10.2 Two-dimensional (2-D) SDS-PAGE

The 2-D non-equilibrium electrophoretic procedure used in these

studies was essentially that of O'Farrell et al. (1977) with slight modifications as described by Haarr and Marsden (1981). Monolayers from 30mm petri dishes were resuspended in 0.3ml H_2O. Cells were then lysed by three cycles of freezing and thawing, each followed by 1min. sonication (Section 2.2) at +4°C. The DNA and RNA of these lysed cells were degraded by adding to each 0.3ml of sample, 0.25ml 30mM Tris, pH7.5, 30mM $MgCl_2$ containing 30 ug/ml DNase 1 and 30 ug/ml RNase A and incubating the mixture at +4°C for 30min. Urea, NP40 and ampholines were added to give final concentrations of 9.2M, 2% and 2%, respectively. At this stage the samples were ready for non-equilibrium pH gradient electrophoresis (NEPHGE) and were either used immediately or were stored at -70°C.

The NEPHGE gel mixture was composed of 9M urea, 2% NP40, 2% ampholines (pH 3.5-10) and 3% acrylamide and 1.62% N,N'-methylenebisacrylamide). Polymerisation was achieved by the addition of ammonium persulphate (0.17%) and TEMED (0.12%) to gel solutions before they were poured into glass tubes with an internal diameter of 2mm. The mixture was overlayed with water. The length of the polymerised gel was 8cm. 5-20ul of the sample prepared as described above was added to the NEPHGE gel and electrophoresed for 3h at 550V. The buffer in the upper reservoir was 0.01M phosphoric acid while the lower reservoir contained 0.02M NaOH (degassed).

After electrophoresis, the seal between the NEPHGE gel and glass was broken with a syringe needle and the gel was gently squeezed from the tube. The gel was then boiled for 10min. in 0.0625M Tris, pH6.8, 2.3% SDS, 0.07M dithiothreitol, 10% glycerol to denature the proteins, the gels were then washed twice in the same buffer with the addition of 1% agarose. For the second dimension, the NEPHGE gel was placed horizontally across the stacking gel of a slab gradient gel and embedded

therein in 1% agarose. The second dimensional electrophoresis was performed as for one-dimensional SDS-PAGE (Section 1.10.1).

2.11 Tryptic peptide mapping of polypeptides

2.11.1 Preparation of tryptic peptides

Polypeptides to be tryptic peptide mapped were first separated on a 10% single concentration SDS-polyacrylamide gel (Section 2.10.1). The gels were dried immediately after electrophoresis. Four radioactive ink marker spots were placed at the corners of the gel which was then exposed to X-ray film (see Section 2.12). Gels were aligned with their autoradiographs by the radioactive spots. Slices of gel containing the polypeptides to be digested were excised by cutting through the autoradiograph into the underlying gel.

Polypeptides were eluted from the gel slices by the method of Anderson et al. (1973). Briefly, gel slices were packed into a column containing electrophoresis buffer (0.025M Tris, 0.19M glycine, 0.1% SDS) and carrier protein (5ug of BSA per column). A small dialysis sac containing about 300ul of electrophoresis buffer was attached to the bottom of the column and the polypeptides were electrophoresed at 100V for 16h at +4^{o}C into the sac.

After electrophoresis the polypeptides were desalted by passage through a prepacked PD10 column (9ml bed volume) containing sephadex G-25 (Pharmacia Laboratories), then lyophilised to dryness. SDS was removed by the procedures used by Henderson et al. (1979). Briefly, the lyophilised polypeptides were resuspended in a solution of acetone, triethylamine, acetic acid, water (85:5:5:5) and incubated at +4^{o}C for 1h. This treatment precipitated the polypeptides while solubilising the SDS. Precipitated polypeptides were pelleted by centrifugation at 13000 rpm for 10min. in a microfuge and washed (by centrifugation at 13000 rpm for 10min.) with the

acetone, triethylamine, acetic acid, water solution, then two more times with acetone only. After the last centrifugation step, the pellet containing the polypeptides was resuspended in 100ul of a 1% solution of ammonium bicarbonate containing 5ug of TPCK-treated trypsin. Digestion was performed for 16h at 37°C, then a further 5ug of TPCK-treated trypsin was added. After a further 4h, the peptides were lyophilised to dryness.

The peptides were oxidised with performic acid (Hirs, 1967) in the following manner: Performic acid was generated by incubating 0.5ml hydrogen peroxide (30 volumes) with 10ml formic acid for 2h. Then to each pellet was added in turn with thorough mixing 100ul of performic acid, 25ul methanol and 40ul performic acid. The mixture was incubated at room temperature for 2.5h when 8ml of water was added to dilute the performic acid. The solution was then lyophilised by freeze-drying.

2.11.2 Separation of tryptic peptides

The peptides were taken up in about 5ul of pH2.1 electrophoresis buffer (glacial acetic acid, formic acid, water; 8:2:90) and applied, 1ul at a time, to a spot 4cm from each of two adjacent edges of a thin-layer cellulose chromatogram (Eastman Kodak). After each application, the spot was dried in a current of cold air. The peptides were electrophoresed for 45min. at 600V, after which time the chromatogram was dried in a current of cold air. Ascending chromatography was performed in a direction perpendicular to that of electrophoresis in 80ml buffer containing H_2O, butan-1-ol, pyridine, acetic acid; 24:30:20:6). The chromatogram was then dried in a current of hot air then sprayed with En^{3}Hance (New England Nuclear) before being exposed to X-ray film (Section 2.12).

2.12 Autoradiography and fluorography

Gels were fixed for 1h in methanol:water:acetic acid (50:50:7)

followed by several washes in methanol:water:acetic acid (5:88:7). For autoradiography, gels were dried under vacuum onto a sheet of filter paper (Whatman grade 182) and exposed to Agfa Scopix CR3NIF at room temperature. For fluorography, fixed and washed gels were placed in three volumes of En^{3}Hance solution (New England Nuclear, Boston, Mass, USA), shaken gently for 1h and then washed twice for 1h in 10 volumes of water before being dried under vacuum as above. Dried gels were exposed to XS-1 film at -70°C.

2.13 Estimation of the amount of protein in individual bands

Fluorographs were scanned with a Joyce-Loebl double-beam scanning microdensitometer. Peaks corresponding to the polypeptides under investigation were cut out of the densitometer scan and weighed.

2.14 Polypeptide nomenclature

For glycoproteins, the nomenclature agreed upon at the Eighth Herpes Simplex Virus Workshop held at Oxford (1983) has been used. This is discussed in detail in Section 1.16. Briefly, glycoproteins are designated by an alphabetical letter. The prefix "g" indicates the mature form of the glycoprotein. Where a glycoprotein also has a prefix "p", the precursor of the glycoprotein is indicated. Where it is not possible to or where it is not wished to distinguish between different forms of a glycoprotein, the prefix is written out in full, e.g. glycoprotein B. When glycoproteins are not resolved from each other, the band is indicated by the names of the glycoproteins likely to be present in it, e.g. glycoprotein B/C. The correlation between this nomenclature and that used by Marsden et al. (1976, 1978) has been discussed by Haarr and Marsden (1981).

The series of spots on two-dimensional gels previously designated pgA to gA (Haarr and Marsden, 1981; Palfreyman et al., 1983) have been

renamed in this thesis since several studies have shown gA and gB to be antigenically related (Eberle and Courtney, 1980b; Pereira et al., 1981; Palfreyman et al., 1983). The nomenclature agreed at the 1983 Oxford meeting makes no provision for multiple fully processed forms of the same core glycoprotein. It is proposed here to make use of their different apparent MWs, as was agreed for precursor forms, and to rename gA and gB, gB_{118} and gB_{122} respectively when it is necessary to differentiate between them.

RESULTS

RESULTS

SECTION A: SULPHATED GLYCOPROTEINS INDUCED BY HERPES SIMPLEX VIRUS

3.1 Prosthetic groups of HSV glycoproteins

3.1.1 Incorporation of ^{35}S-inorganic sulphate into HSV-infected cells

To determine if sulphate was incorporated into HSV-induced glycoproteins, BHK cells infected with HSV were labelled with ^{35}S-inorganic sulphate. Initial experiments showed that incorporation of the label into trichloroacetic acid (TCA)-precipitable material in both uninfected and infected cells was higher if the cells were grown in sulphate-free rather than normal medium. To test whether inorganic sulphate is incorporated into the same major macromolecules in normal or sulphate-free medium, both uninfected and infected cells were labelled with 500uCi/ml ^{35}S-inorganic sulphate in either medium, and the same proportion of cells from each dish were resolved by SDS-PAGE. Fig. 24 shows that using either medium, the label was incorporated in uninfected cells into macromolecules of heterogeneous size (lanes 4, 5). After infection with HSV-1 or HSV-2, incorporation was dramatically reduced and the profile was altered (lanes 1, 2, 3, 6, 7, 8). Lanes 1 and 8 are longer exposures of lanes 2 and 6 respectively. Comparison of lane 1 with lane 3 and lane 7 with lane 8 shows that the increased incorporation in sulphate-free medium was into the same major macromolecules as in normal medium. Subsequent experiments were therefore performed in sulphate-free media.

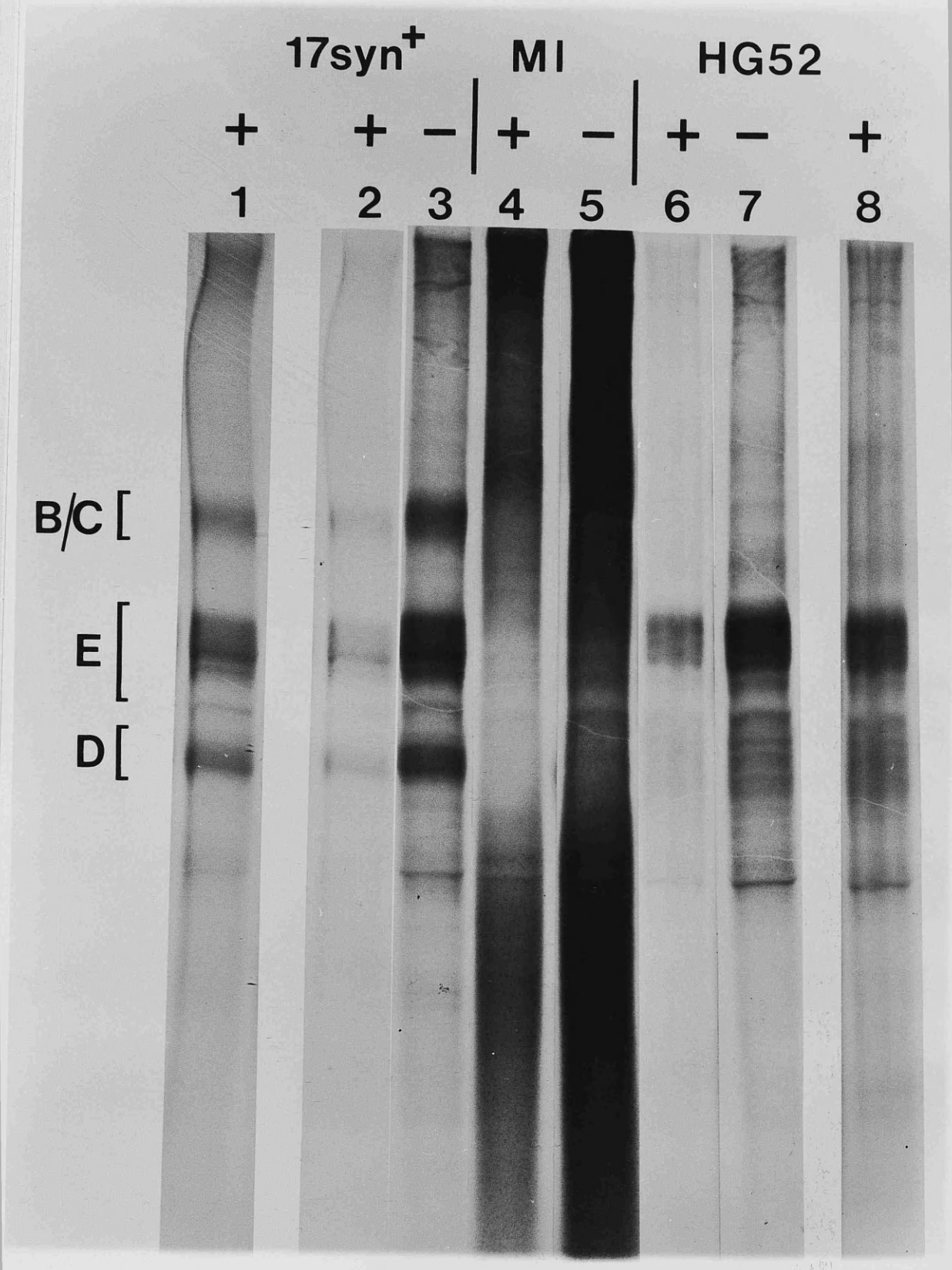

17syn+
MI
HG52
+
+
−
+
−
+
−
+
1
2
3
4
5
6
7
8
B/C
E
D

FIGURE 24

Autoradiograph of sulphated macromolecules synthesised in cells infected with 17 syn^+ (HSV-1), HG52 (HSV-2) and in mock-infected (MI) cells. Cells were labelled from 2-24h after infection with ^{35}S-inorganic sulphate either in the presence of normal concentrations of inorganic sulphate (+) or in the absence or inorganic sulphate (-). The polypeptides in this and subsequent figures were resolved on 5%-12.5% SDS-polyacrylamide gels, unless otherwise stated. Lanes 1 and 8 are from an autoradiograph which was exposed for approximately six times longer than that from which lanes 2 to 6 were taken.

* The bands migrating at 25K and 24K were not consistently observed and remain uncharacterised.

3.1.2 Virus-induced polypeptides which label with inorganic sulphate

Since the glycoproteins of many viruses are known to be sulphated, the mobility, on SDS-polyacrylamide gels, of polypeptides labelled from 5h to 7h PI with ^{35}S-inorganic sulphate or [^{3}H]-mannose were compared (fig. 25). In cells infected with either HSV-1 or HSV-2, a major sulphated moiety was induced which, from data presented later, can be identified as gE. In addition, in HSV-1 infected cells, less intense sulphated bands co-migrated with glycoproteins B/C, D and polypeptides of apparent MW 32000, 34000 and 35000 (32K, 34K and 35K). The 32K, 34K, and 35K polypeptides can be detected in fig. 25 but are more clearly seen in later figures (MWs were determined from the data shown in fig. 29).* The only less-intense sulphated band consistently seen in HSV-2-infected cells co-migrated with glycoprotein D although sulphate label co-migrating with glycoproteins B/C was occasionally observed as were a number of other bands (e.g. fig. 25). The relative intensities of glycoproteins B/C, D and E based on visual estimates from the autoradiographs of five experiments are shown in Table 10. Glycoproteins B/C and D were generally more heavily labelled during a 2-24h label than during a pulse of 1h to 2h duration, an observation which will be discussed later. Fig. 25 also shows that for glycoprotein D the sulphate was associated only with the more fully processed forms and not the precursor (pgD).

To determine whether glycoproteins B, C and Y are sulphated, HFL cells were infected with HSV-1 or were mock-infected (MI) and proteins labelled with ^{35}S-inorganic sulphate or with [^{3}H-mannose]. The labelled proteins were separated by 2-D gel electrophoresis (fig. 26). Identification of the major glycoproteins, fig. 26 (a) was based on immunoprecipitation with monoclonal antibodies (Palfreyman et al., 1983). pgB117 and pgB107 are two mannose-containing electrophoretically well separated early intermediates of glycoprotein B. Fig. 26 (b) shows sulphated, virus-induced

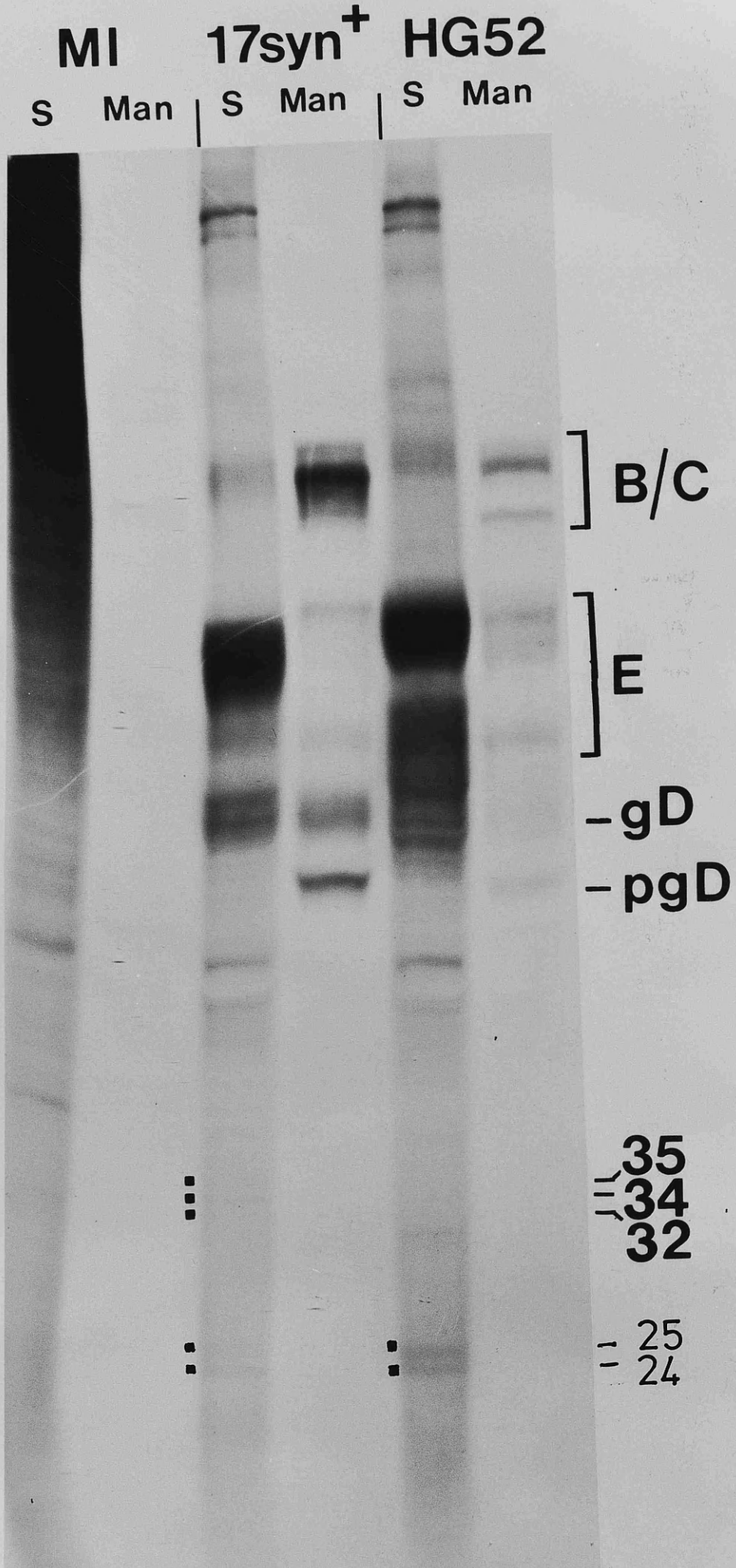

MI
17syn+
HG52
S
Man
S
Man
S
Man
B/C
E
gD
pgD
35
34
32
25
24

FIGURE 25

Comparison of the polypeptides in infected cells labelled with either [^{3}H]-mannose (man) or ^{35}S-inorganic sulphate (S) in PBS + 1% non-essential amino acids. Cells were infected with either 17 syn^{+} (HSV-1) or HG52 (HSV-2), or were mock infected (MI). They were labelled from 5 to 7h post-infection.

TABLE 10

HSV-sulphated glycoproteins

Labelling time	Serotype	Glycoprotein	Relative intensity*
Continuous from 2h - 24h Post-infection	HSV-1	B/C	+
		E	+++
		D	++
	HSV-2	B/C	δ
		E	+++
		D	+
Pulsed at 5h Post-infection for 1h - 2h duration	HSV-1	B/C	δ
		E	+++
		D	+
	HSV-2	B/C	δ
		E	+++
		D	δ

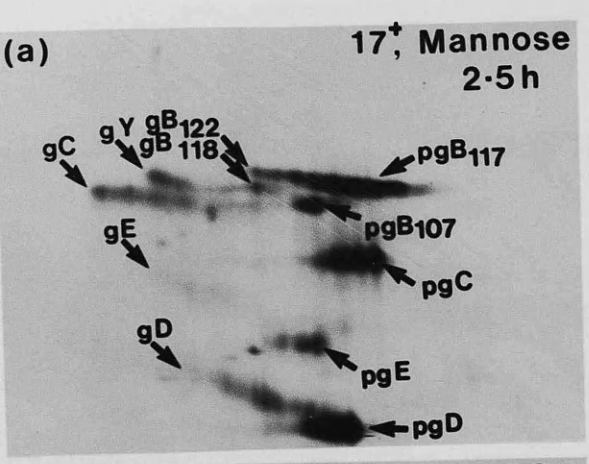
* Four categories of intensity are used. However, the intensity of a particular band varies plus or minus one category from experiment to experiment:

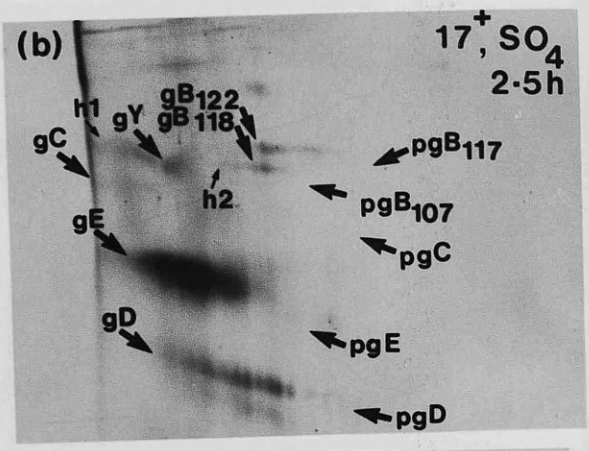
+++ very intense band

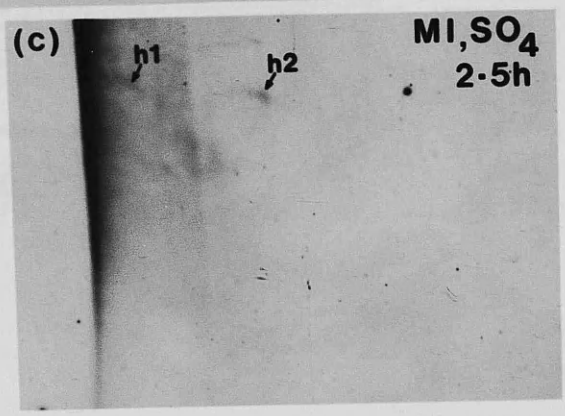
++ strong band

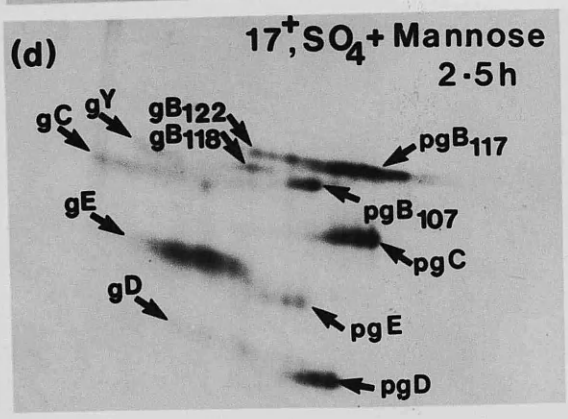
+ minor band

δ trace amounts sometimes seen


(a)
17+, Mannose
2·5h
gC
gY
gB122
gB 118
pgB117
pgB107
gE
pgC
gD
pgE
pgD


(b)
17+, SO4
2·5h
h1
gY
gB122
gB 118
gC
pgB117
h2
pgB107
gE
pgC
gD
pgE
pgD


(c)
MI, SO4
2·5h
h1
h2


(d)
17+, SO4+ Mannose
2·5h
gC
gY
gB122
gB118
pgB117
pgB107
gE
pgC
gD
pgE
pgD

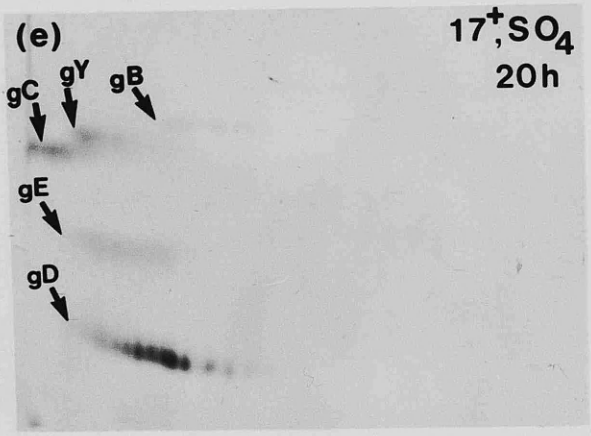

(e)
17+, SO4
20h
gC
gY
gB
gE
gD

FIGURE 26

Sulphation of glycoproteins B, C, D, E and Y in HFL cells infected with HSV-1 strain 17 syn^+. HFL cells were mock-infected (MI; c) or infected with 17 syn^+ (a, b, d, e) and labelled with either [^{3}H]-mannose (a) or ^{35}S-inorganic sulphate (b, e) from 5-7.5h (a, b, c, d; 2.5h) or from 2-22h (e; 20h) after infection. (d) Separation of a mixture of ^{35}S-inorganic sulphate and [^{3}H]-mannose-labelled polypeptides. Polypeptides were resolved by 2D electrophoresis (NEPHGE). For the 2D gel shown in this figure and fig. 32, Ampholines pH 3.5 to 10 were used for the first dimension and 5-12.5% SDS-polyacrylamide gel for the second dimension. The acidic end of the NEPHGE gel is on the left. The fluorographs have been cut to show only polypeptides of apparent MW between about 45,000 and 130,000.

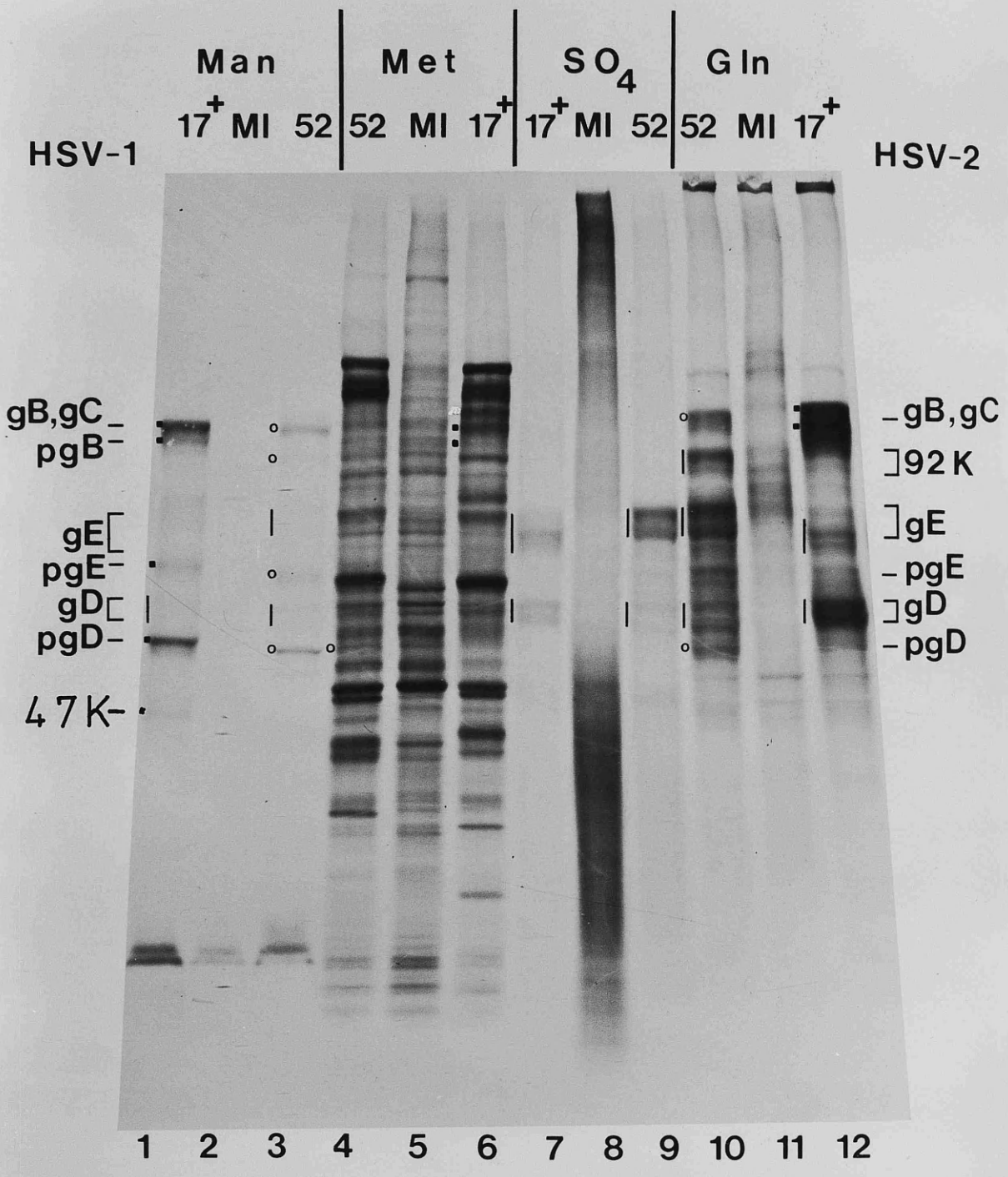

Man
Met
SO4
Gln
17+ MI 52 52 MI 17+ 17+ MI 52 52 MI 17+
HSV-1
HSV-2
gB,gC
pgB
gE
pgE
gD
pgD
47K
gB,gC
92K
gE
pgE
gD
pgD
1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 27

Comparison of the polypeptides of infected and uninfected cells labelled with [^{3}H]-mannose (Man), [^{35}S]-methionine (Met), ^{35}S-inorganic sulphate (SO_4) or [^{14}C]-glucosamine (Gln). Cells were infected with either HSV-1 strain 17 syn^{+} (17^{+}) or HSV-2 strain HG52 (52) or were mock-infected (MI). Labelling with mannose was from 5 to 6.5h after infection and with the other isotopes from 2 to 24h after infection. In this figure the symbols for HSV-1 glycoproteins (■) and HSV-2 glycoproteins (o) are placed adjacent and to the left of the appropriate lane.

is further characterised in Sections 3.9 - 3.13. Other polypeptides not indicated on this lane may be precursor or processed forms of the identified glycoproteins but are as yet unidentified species. [^{14}C]-glucosamine (lane 12) is incorporated in HSV-1 infected cells most intensely into gB/C, pgB and gD, while pgD is relatively poorly labelled. Polypeptides migrating in the vicinity of gE are also poorly labelled appearing as a triplet migrating in part slightly faster than the HSV-1 inorganic sulphate-labelled gE (lane 7). Once again polypeptides not indicated, cannot be identified with any degree of certainty.

3.2 Sulphated polypeptides which are secreted

3.2.1 Identification of sulphated polypeptides which are secreted

Secreted polypeptides were found in the supernatant after centrifuging the growth medium at 35000 x g for 2h (Methods, Section 2.5). This procedure removed the majority of virions from the growth medium whilst leaving behind most of the TCA-precipitable counts (Table 11). The table shows that the yield of supernatant virus was not reduced by the absence of inorganic sulphate from the growth medium. It also shows that the fraction of TCA-precipitable counts remaining in the growth medium after centrifugation was essentially unaffected by the presence in, or absence from, the growth medium of inorganic sulphate. These observations further justify the use of sulphate-free medium. Fig. 28 shows intracellular polypeptides and polypeptides found in the extracellular growth medium before and after centrifugation. In this experiment, cells infected with 17 syn$^+$ were labelled with ^{35}S-inorganic sulphate or [14C]-glucosamine (which, like mannose, serves to indicate the positions of glycoproteins). Most of the sulphated glycoproteins B/C and E were removed from the extracellular growth medium by centrifugation, suggesting that they are virion-bound or exist in an aggregated form. On the other hand, most of the sulphated protein which co-migrates with gD

TABLE 11

Removal of virus from the growth medium of infected cells

Virus	Medium	Virus titre in growth media			^{35}S TCA-precipitable counts in growth media		
		Before centrifuging	After centrifuging	Fraction remaining	Before centrifuging	After centrifuging	Fraction remaining
17 syn$^+$	Normal	1.8×10^8	$<1.0 \times 10^5$	<0.1%	2450	2133	86%
	Sulphate-free	5.1×10^8	5.2×10^6	1.0%	7234	5600	77%
HG52	Normal	3.1×10^6	$<1.0 \times 10^5$	<4.0%	2376	2238	94%
	Sulphate-free	6.3×10^6	$<1.0 \times 10^5$	<2.0%	5037	4270	84%

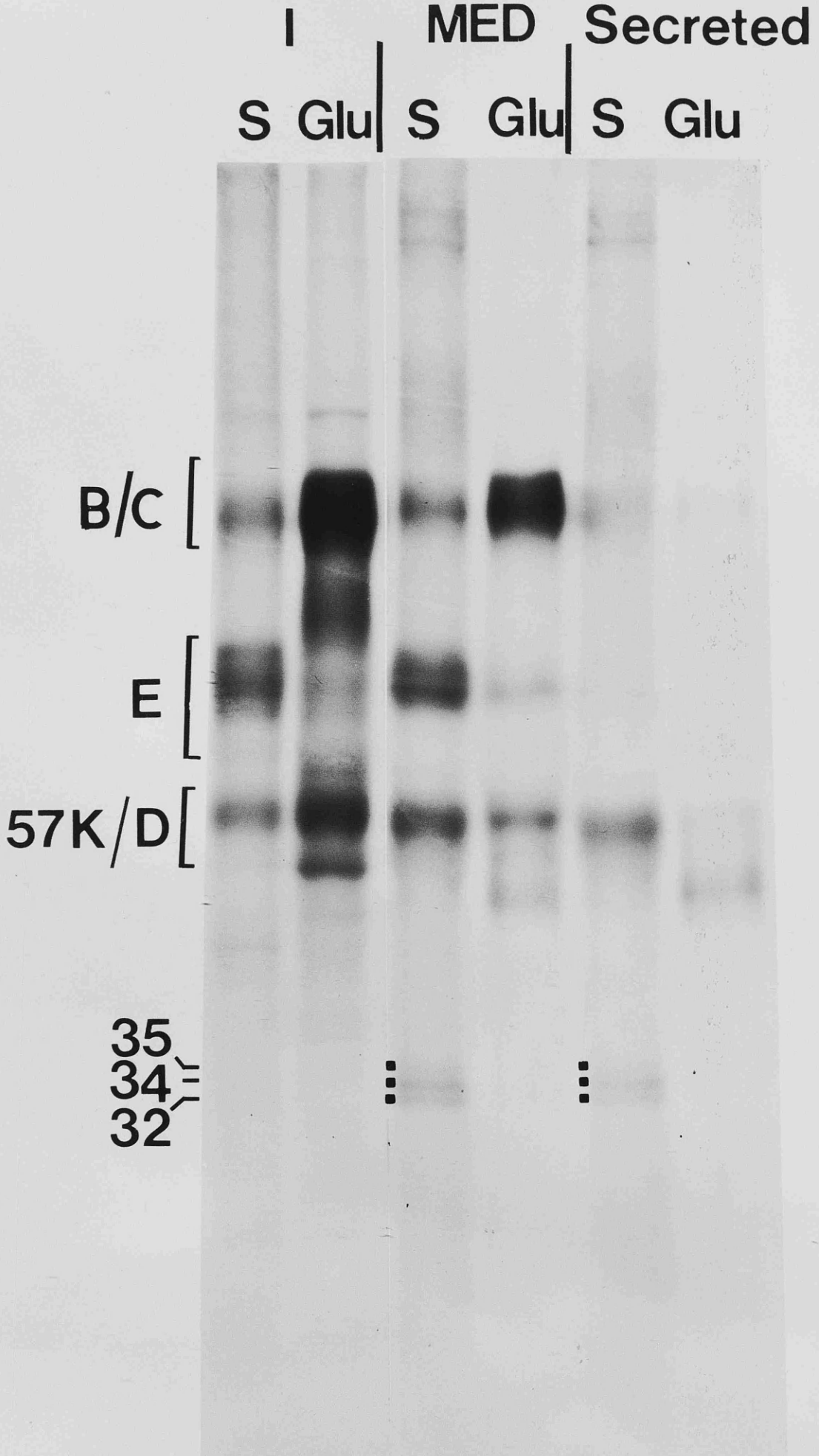

I
MED
Secreted
S
Glu
S
Glu
S
Glu
B/C
E
57K/D
35
34
32

FIGURE 28

Comparison of intracellular polypeptides (I) and polypeptides in the growth medium before (Med) and after (Secreted) centrifugation at 35000g for 2h. Polypeptides of cells infected with HSV-1 strain 17 syn^+ were labelled in sulphate-free medium from 2 to 24h after infection with either ^{35}S-inorganic sulphate (S) or [^{14}C]-glucosamine (Glu).

was not removed, suggesting that it is largely secreted and is soluble in the growth medium. Also secreted were the sulphated polypeptides of apparent MWs 32000 and 34000. In addition, lower amounts of a polypeptide of apparent MW 35000 and small amounts of a sulphated glycoprotein of apparent MW 47000 (more clearly seen on Fig. 42, lane 4) were frequently observed to be secreted.

3.2.2 Apparent MW of sulphated polypeptides

The previously referred to apparent MW of the intracellular and secreted sulphated polypeptides were determined by comparing their mobility (on 5% to 12.5% SDS-polyacrylamide gels) with those of [^{35}S]-methionine-labelled intracellular polypeptides (fig. 29). The strongest virus-induced sulphated band secreted from cells infected with HG52 migrated in the vicinity of glycoprotein D with an apparent MW of 57000 to 63000 (also fig. 42). A band of similar apparent MW (47000 to 57000) was secreted from cells infected with 17 syn$^+$ and, in addition, the 32K and 34K polypeptides were very prominent with lesser amounts of the 35K polypeptide. Additional sulphated bands observed in this and other figures co-migrated with host sulphated bands (fig. 42).

3.2.3 Quantification of the amount of sulphated 32K, 34K and 35K polypeptides which are secreted from HSV-1-infected cells

Figs. 28 and 29 show that in comparison with the other sulphated glycoproteins, a polypeptide co-migrating with gD and the 32K, 34K and 35K polypeptides are preferentially secreted. To quantify the ratio of the amounts of 32K, 34K and 35K secreted to that synthesised, densitometer scans were made of autoradiographs of intracellular and secreted sulphated polypeptides from experiments similar to those shown in fig. 29. From a knowledge of the volumes in which the proteins were harvested and the volumes loaded onto gels, the values shown in Table 12 were calculated.

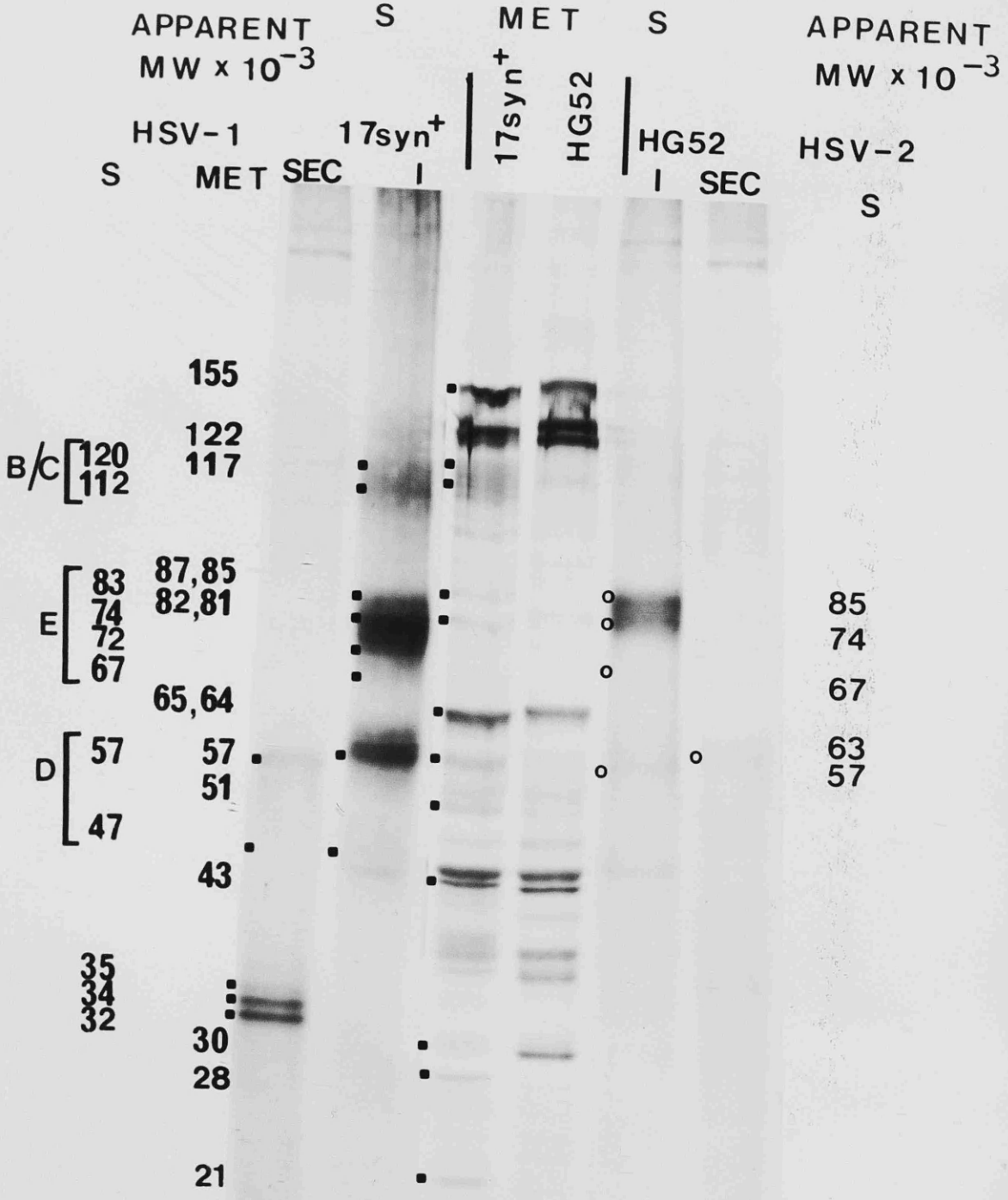

APPARENT MW x 10⁻³
S
MET
S
APPARENT MW x 10⁻³
HSV-1
17syn+
17syn+
HG52
HG52
HSV-2
S
MET
SEC
I
I
SEC
S
155
122
117
120
112
B/C
87,85
83
82,81
74
72
67
E
85
74
67
65,64
57
51
47
D
57
63
57
43
35
34
32
30
28
21

FIGURE 29

Comparison of the mobilities of virus-induced polypeptides labelled with either [^{35}S]-methionine (Met) or ^{35}S-inorganic sulphate (S) in cells infected with HSV-1 strain 17 syn^{+} or HSV-2 strain HG52. Both intracellular and secreted (Sec) sulphated polypeptides are shown.

TABLE 12

Fraction of sulphated 32K, 34K, 35K polypeptides which are secreted from HSV-1 infected cells

Experiment	Cell	Fraction of sulphated 32K, 34K, 35K polypeptides which are secreted*
1	BHK	1.00
2	BHK	0.86
3	BHK	0.97
4	BHK	0.94
5	HFL	0.93

* $$\frac{\text{32K, 34K, 35K (secreted)}}{\text{32K, 34K, 35K (secreted)} + \text{32K, 34K, 35K (intracellular)}}$$

where 32K, 34K, 35K (secreted) =

$$\text{area of 32K, 34K, 35K secreted peak} \times \frac{\text{volume in which polypeptides harvested}}{\text{volume loaded onto gel}}$$

the 32K, 34K, 35K (intracellular) is similarly calculated

About 95% of the 32K, 34K and 35K polypeptides which were made were secreted. Similar amounts were secreted from both BHK and HFL cells.

It is clear from fig. 42 that the sulphated polypeptides form only a subset of the total polypeptides secreted from 17 syn+-infected cells (compare lane 4 with lanes 3 and 9). For example, in addition to the 32K, 34K and 35K, bands (lane 9) polypeptides having apparent MWs of 112000 to 120000, 57000, 47000, 45000 and 40000 were released from [^{14}C]-glucosamine-labelled cells infected with 17 syn+. From densitometer scans made of the autoradiographs shown in this figure and of autoradiographs from other similar experiments, it was calculated that the 32K, 34K and 35K polypeptides comprise only about 5% of [^{14}C]-glucosamine-labelled polypeptides and about 10% of [^{35}S]-methionine-labelled polypeptides secreted from 17 syn+-infected cells.

3.3 Additional evidence that the sulphated macromolecules are virus-induced

That the sulphated polypeptides are virus-induced is suggested by the observations that at the NPT (38.5^{o}C) the mutant tsK which is blocked very early in infection (Preston, 1979a) did not induce them in significant amounts (fig. 30). The sulphated polypeptides of apparent MWs 32K, 34K and 35K also appeared to be virus-induced as they were not seen in cells infected at 38.5^{o}C with tsK. However, the high MW intracellular sulphated moiety which migrated near the top of the resolving gel (figs. 25 and 26) is probably of host origin since it was present in the 38.5^{o}C infection with tsK. It is recognised that the high MW secreted polypeptides migrating at the top of this and other figures (figs. 28 and 29), are shut off by tsK at 38.5^{o}C. This is probably a demonstration of the observations of Fenwick and Walker (1978) and Nishioka and Silverstein (1978b) that host protein synthesis is shut off by a virion component and/or an immediate-early HSV-induced polypeptide. These high MW polypeptides

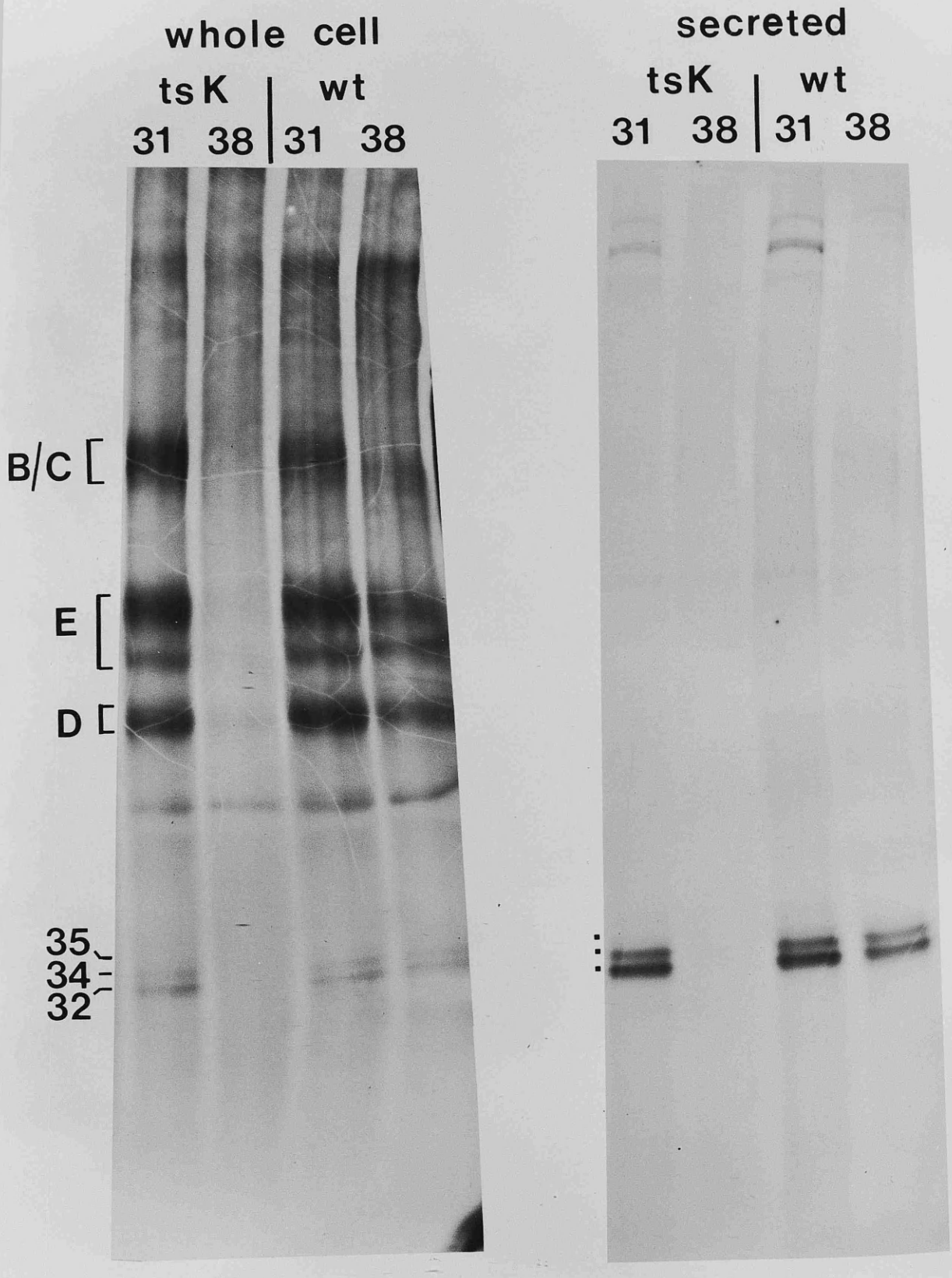

whole cell
tsK
wt
31
38
31
38
secreted
tsK
wt
31
38
31
38
B/C
E
D
35
34
32

FIGURE 30

Failure of tsK to induce either sulphated polypeptides in the whole infected cell (whole cell) or sulphated secreted polypeptides at the non-permissive temperature. Cells were infected with either 17 syn^+ ts^+ (wt) or 17 syn^+ tsK (tsK) at 31°C (the permissive temperature) and 38.5°C (the non-permissive temperature) and labelled from 2-24h post-infection with ^{35}S-labelled inorganic sulphate.

can be seen to co-migrate with host sulphated polypeptides (figs. 38 and 42) and host [^{14}C]-glucosamine labelled polypeptides (fig. 42).

3.4 Identification of the major sulphated HSV-induced glycoprotein as glycoprotein E

Baucke and Spear (1979) identified an HSV-induced glycoprotein (designated gE) which had affinity for the Fc region of IgG. It was tested whether any of the HSV-induced sulphated glycoproteins possessed this affinity. Fc-affinity columns consisting of rabbit antibody bound to an antigen (BSA) which was itself covalently coupled to sepharose 4B were prepared as described by Baucke and Spear (1979). Control columns lacked rabbit antibody. Equal volumes of an extract of cells infected with either 17 syn+ or HG52 and labelled with ^{35}S-inorganic sulphate were applied to the affinity or control columns and eluted as described. The results are presented in fig. 31. The major sulphated glycoprotein in cells infected with 17 syn+ was more strongly bound by the Fc-affinity column (lane 2) than by the control column (lane 3) indicating identity with gE, the Fc-binding glycoprotein. The major sulphated glycoprotein induced by HG52 also bound more strongly to the Fc-affinity column (lane 5) than to the control column (lane 6). Although it is not possible to tell whether binding was selective as it was the only major sulphated protein applied to the column, the result suggests that the major sulphated glycoprotein of HG52 also has Fc-binding activity. (In this experiment, some of the HG52-infected cell extract was lost and fewer counts were loaded onto both Fc-affinity and control columns).

The major intracellular sulphated polypeptide in 17 syn+-infected cells had an apparent MW of 72000 to 83000 with a fainter band at 67000. In HG52-infected cells, the intracellular polypeptide of major intensity had an apparent MW of 74000 to 85000 and again there was a minor band at

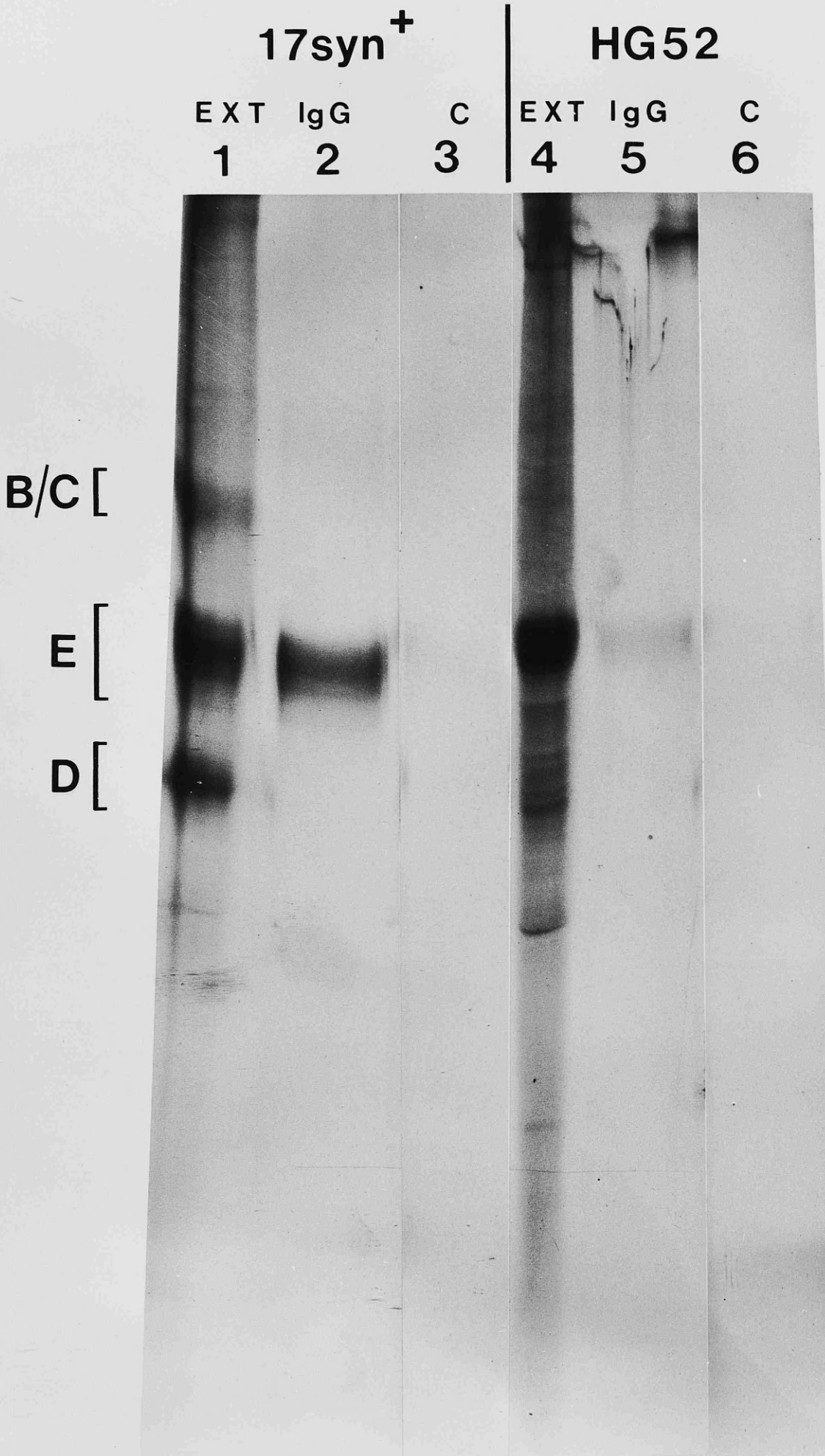

17syn+
HG52
EXT
IgG
C
EXT
IgG
C
1
2
3
4
5
6
B/C
E
D

FIGURE 31

Fc-affinity chromatography of HSV-induced sulphated glycoproteins. Equal volumes of extracts (EXT) of cells infected with HSV-1 strain 17 syn+ or HSV-2 strain HG52 and labelled with ^{35}S-inorganic sulphate were applied to either IgG-BSA-sepharose (IgG) or control BSA-sepharose (C) columns. After extensive washing, bound polypeptides were eluted with 3M-potassium thiocyanate as described by Baucke and Spear (1979). The bound and eluted polypeptides together with the infected cell extracts were resolved by SDS-PAGE. All lanes come from one autoradiograph of a single gel, but some intervening irrelevant lanes have been removed. All lanes of the autoradiograph were exposed to the gel for equal times.

67000 (not clearly seen in this figure, but regularly seen in a pulse, e.g. fig. 25). These MWs are almost identical to the MW range of 65000 to 80000 determined by Baucke and Spear (1979) for glycoprotein E and provide supportive evidence that the sulphated glycoprotein is glycoprotein E. The apparent MWs of other intracellular sulphated polypeptides are indicated on fig. 29.

3.5 Evidence suggesting that gE and gY contain N-linked oligosaccharides

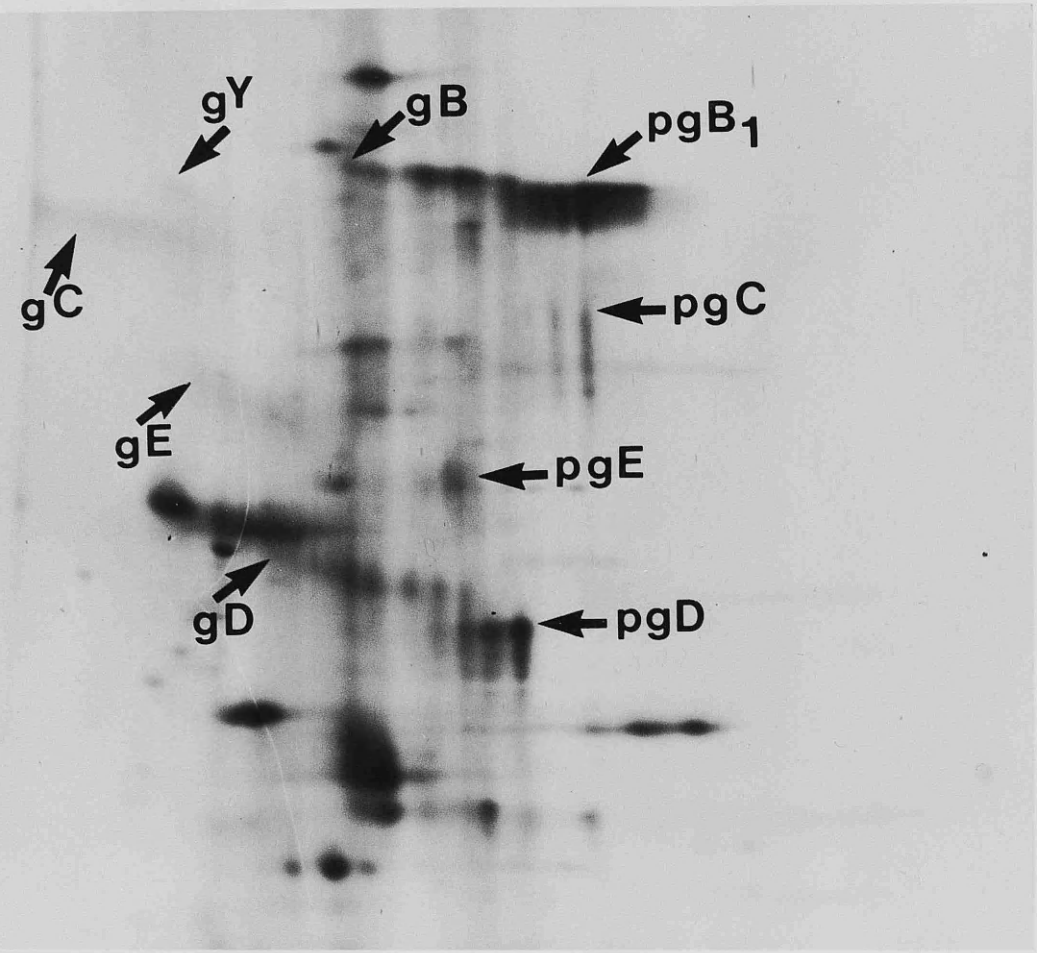
The drug tunicamycin was used to investigate whether glycoproteins E and Y contain N-linked oligosaccharides. HFL cells were infected with HSV-1 strain 17 syn+ either in the presence or absence of tunicamycin and labelled with [^{35}S]-methionine from 5h to 8h after infection. Labelled polypeptides were analysed by 2-D gel electrophoresis (fig. 32). In the absence of the drug, the fully mature forms of the major glycoproteins (gB, gC, gD and gE) were synthesised, as was gY. In the presence of tunicamycin, none were made, suggesting that all the glycoproteins and, in particular, gE and gY, contain N-linked oligosaccharides (the positions to which the fully processed glycoproteins would migrate if they were present are shown). Several novel polypeptides indicated by small arrows accumulated in the presence of the drug. They form three groups designated 1, 2 and 3 having apparent MWs of 105000, 75000 and 47000 respectively and probably correspond to those of MWs 110000, 80000 and 50000 previously observed (Pizer et al., 1980) to accumulate in the presence of the drug.

3.6 Nature of the linkage of inorganic sulphate to HSV glycoproteins

HFL cells were either mock-infected or infected with HSV-1 and labelled with ^{35}S-inorganic sulphate, [^{35}S]-methionine and [^{3}H]-mannose. Infection and labelling were performed in both the absence and in the

NO DRUG

+ TUNICAMYCIN



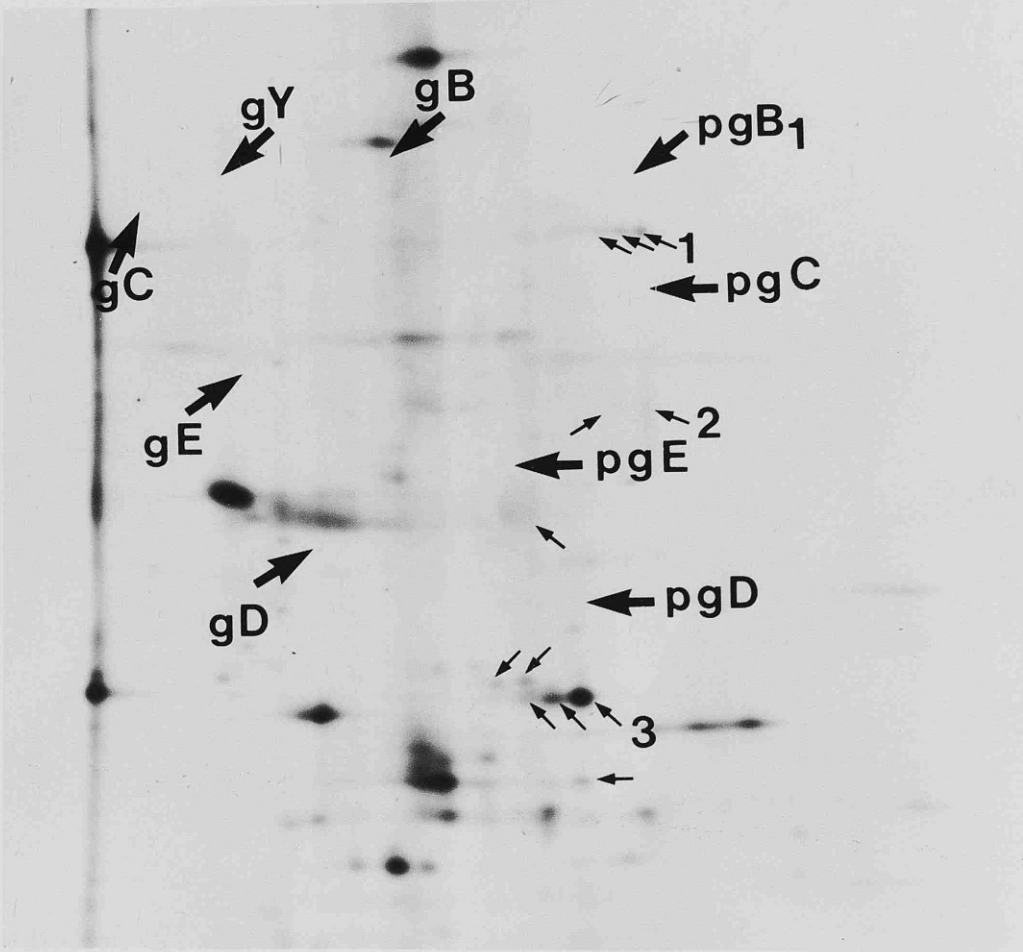


FIGURE 32

Effect of tunicamycin on polypeptide synthesis in HFL cells infected with HSV-1 strain 17 syn^+. Cells were labelled from 5 to 8h after infection with [^{35}S] methionine and polypeptides were separated on 2D gels as for fig. 26. The left-hand fluorograph shows polypeptides synthesised in the absence of the drug; the right-hand fluorograph shows polypeptides synthesised in infected cells treated with tunicamycin. Large arrows indicate the major HSV-1-induced glycoproteins and their precursors or the positions to which they would migrate if they were present. Small arrows designated 1, 2 and 3 indicate the novel HSV-1-infected cell polypeptides synthesised in the presence of the drug.

* Incorporation into other bands – possibly non-viral – is not reduced.

presence of tunicamycin and polypeptides were analysed by SDS-PAGE (fig. 33). Tunicamycin essentially abolished incorporation of [^{3}H]-mannose into HSV-1 glycoproteins (compare lanes 6 and 9). This result confirms the earlier work of Pizer et al. (1980) and suggests that glycoproteins B, C and D contain N-linked oligosaccharides. Evidence suggesting that gE contains N-linked oligosaccharides is provided by the observation that the drug reduced the incorporation of ^{35}S-inorganic sulphate into gE (compare lanes 4 and 7). The drug also prevented incorporation of inorganic sulphate into gB, gC and gD.* However, incorporation of inorganic sulphate into HSV-1-infected cells was not as dramatically reduced by tunicamycin as was incorporation of [^{3}H]-mannose (Table 13). Several bands were still labelled with ^{35}S-inorganic sulphate in infected cells in the presence of the drug, in particular, one of approximate MW 70000 was synthesised. Major [^{35}S]-methionine-labelled polypeptides of apparent MW 105000 and 47000 accumulate in the presence of the drug (compare in fig. 33, lane 8 with lane 5) and correspond to those seen in fig. 32. Results of experiments investigating the origin of the 70K polypeptides will be presented later.

3.7 Physical map location of sulphated polypeptides

3.7.1 Mapping of the major intracellular sulphated glycoprotein

It was a consistent observation that gE induced by HG52 migrated slightly slower in 5% to 12.5% gradient SDS-PAGE gels than did gE induced by 17 syn+ (figs. 24, 25, 27, 29 and 31). This mobility difference enabled a physical map location for the glycoprotein to be obtained. Mock-infected or infected cells grown in sulphate-free medium were labelled with ^{35}S-inorganic sulphate from 5h to 7h PI, a procedure which labelled predominantly gE (Table 10). Recombinants 17+x11r (1), Bx1 (28-1-1) and Bx6 (17-1) induced a type 2 polypeptide whereas RE6, RH6 and

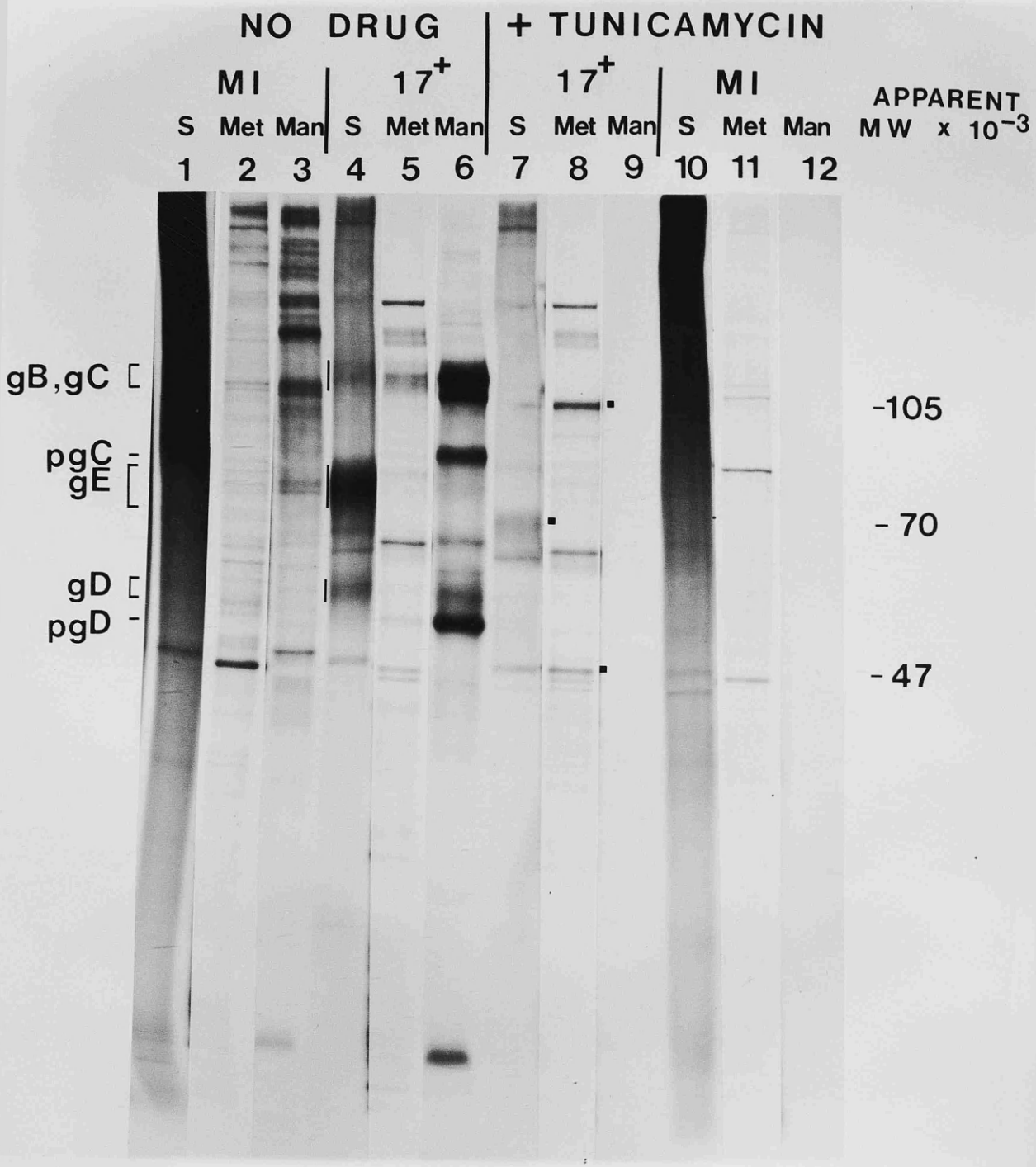

NO DRUG
+ TUNICAMYCIN
MI
17+
17+
MI
S Met Man S Met Man S Met Man S Met Man
1 2 3 4 5 6 7 8 9 10 11 12
APPARENT MW x 10−3
gB,gC
pgC
gE
gD
pgD
-105
-70
-47

FIGURE 33

Effect of tunicamycin on HFL cells infected with HSV-1 strain 17 syn^+. Cells were either infected (17^+; lanes 4 to 9) or were mock-infected (MI; lanes 1, 2, 3, 10, 11, 12) and labelled from 5 to 8h after infection with ^{35}S-inorganic sulphate (S; lanes 1, 4, 7, 10), [^{35}S]-methionine (Met; lanes 2, 5, 8, 11) or D-[2-^{3}H]-mannose (Man, lanes 3, 6, 9, 12).

TABLE 13

Effect of tunicamycin on the incorporation of [^{35}S]-methionine, ^{35}S-inorganic sulphate and [^{3}H]-mannose into HSV-1-infected or mock-infected cells

Type of infection	Radioactive label	TCA*-precipitable counts ($\times 10^{-3}$) incorporated from 5h to 8h post-infection		Percentage of counts incorporated in TM*-treated cells compared with untreated controls
		-TM	+TM	
Mock-infected	[^{35}S]-Met	4650	2240	48.0
	$^{35}SO_4$	514	199	38.8
	[^{3}H]-Mann	156	0.7	0.4
HSV (strain 17 syn^{+})	[^{35}S]-Met	4180	3120	74.5
	$^{35}SO_4$	47	18	37.3
	[^{3}H]-Mann	185	1	0.6

* TCA, Trichloracetic acid; TM, tunicamycin

RE4 induced a type 1 polypeptide (fig. 34). Correlation of these data with the genome structures of these recombinants (fig. 35) gave a map location between 0.832 and 0.950mu of the genome, delimited on the left by the BamHI q'-m' site of Bx1 (28-1-1) and on the right by the XbaI j-i site of RE4 with all data consistent.

Another six intertypic recombinants, each of which contained crossovers in the short region, were used to refine further this location. Fig. 36 shows extracts of MI and infected cells grown in sulphate-free medium and labelled with ^{35}S-inorganic sulphate from 2h to 22h PI. All six recombinants induce gE-1. Correlation of these data with the genome structures of these recombinants (fig. 37) gives a map location delimited on the left-hand side by the HSV-2 BglII q-l site (0.886) and on the right-hand side by the HSV-2 EcoRI n-o site (0.935) with all the data consistent.

Since these studies, the gene encoding glycoprotein E of HSV-1 has been sequenced (McGeoch et al., 1985) and the coding region lies between residues 8693 and 10289 of the U_S component of the HSV genome.

3.7.2 Mapping of the sulphated secreted proteins 32K, 34K and 35K

To gain insight into the origin of the secreted 32K, 34K and 35K proteins, they were mapped using the same set of recombinants that were used for the mapping of gE. The proteins were secreted by recombinants RE4 and RE6, but not by HG52 or recombinants 17+x11^r (1), Bx1 (28-1-1) or Bx6 (17-1) (fig. 38) from which a map position of 0.832 to 0.950mu can be deduced. These co-ordinates are again delimited on the left by Bx1 (28-1-1) and on the right by RE4 with all data consistent.

Using the same second set of recombinants described above, fig. 39 shows that cells infected with each of the six recombinants all secrete the 32K, 34K and 35K proteins, thus giving a map location identical to that obtained for gE which is delimited on the left-hand side by the HSV-2

1
17+ x 11r(1)
2
B x 1(28-1-1)
1
B x 6(17-1)
2
R E6
1
R H6
2
R E4
1
MI
B/C
E
D

FIGURE 34

Mapping of the major intracellular sulphated glycoprotein (glycoprotein E). Fluorograph of sulphated macromolecules induced in mock-infected (MI) cells and in cells infected with the intertypic recombinants $17^{+}x11^{r}$ (1), Bx1 (28-1-1), Bx6 (17-1), RE6, RH6, RE4 and the parental viruses HSV-1 strain 17 syn^{+} and HSV-2 strain HG52. Cells were labelled with ^{35}S-inorganic sulphate from 5 to 7h after infection.

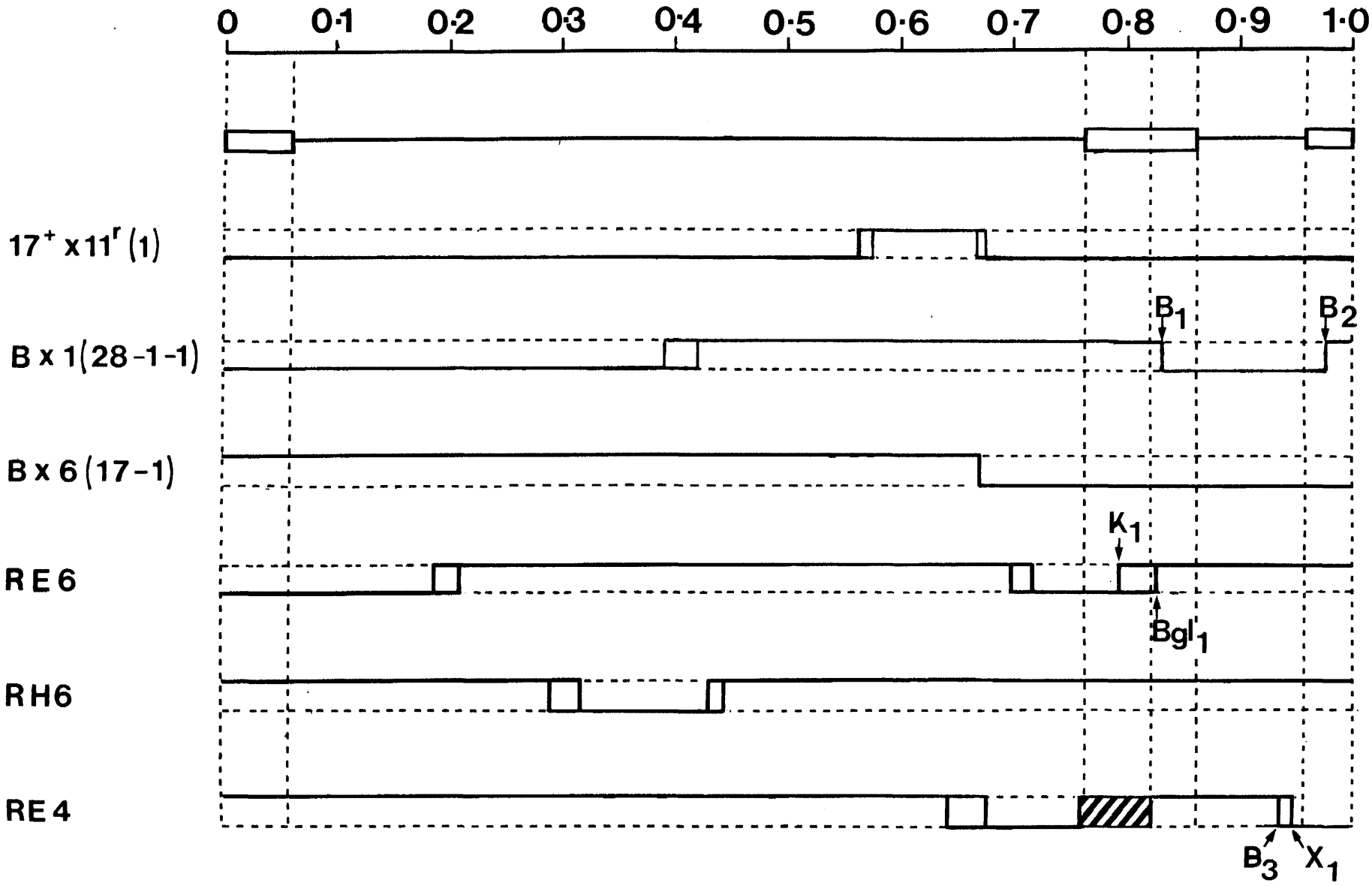

0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
17+ x 11r (1)
B x 1(28-1-1)
B x 6 (17-1)
RE 6
RH6
RE 4
B1
B2
K1
Bgl1
B3
X1

FIGURE 35

Summary of the genome structures of the six recombinants used for the experiment represented in fig. 34. The genome arrangement of HSV-DNA is illustrated in the prototype configuration at the top of the figure, showing the long and short repeat sequences and the long and short unique regions. Vertical dotted lines correspond to the ends of the long and short repeat sequences. Those sequences of the recombinant derived from the type 1 and type 2 parent are represented by a thick continuous line superimposed respectively on the upper and lower of the two horizontal dotted lines. Crossover regions are indicated by one or two vertical lines between the thick continuous horizontal lines. The distance apart of two vertical lines indicates the remaining region of uncertainty for that crossover event. Where the uncertainty is small, the crossover appears as a single vertical line. The units on the top are expressed as a fraction of the genome length. Although RE4 is fixed in the I_S configuration, it is drawn in the prototype configuration, the hatched box represents deleted DNA sequences. There is a transposition of DNA sequences in RE4, of HSV-2 sequences from 0.26-0.28mu into the position of deleted sequences (Davison and Wilkie, 1983). Delimiting restriction sites in the short region of the genome in HSV-1 (↓) and HSV-2 (↑) are designated as follows:

B1, BamHl q-m'; B2, BamHI m'-q; B3, BamHI d'-b'; BglI, BglII i-h; X, XbaI j-i.

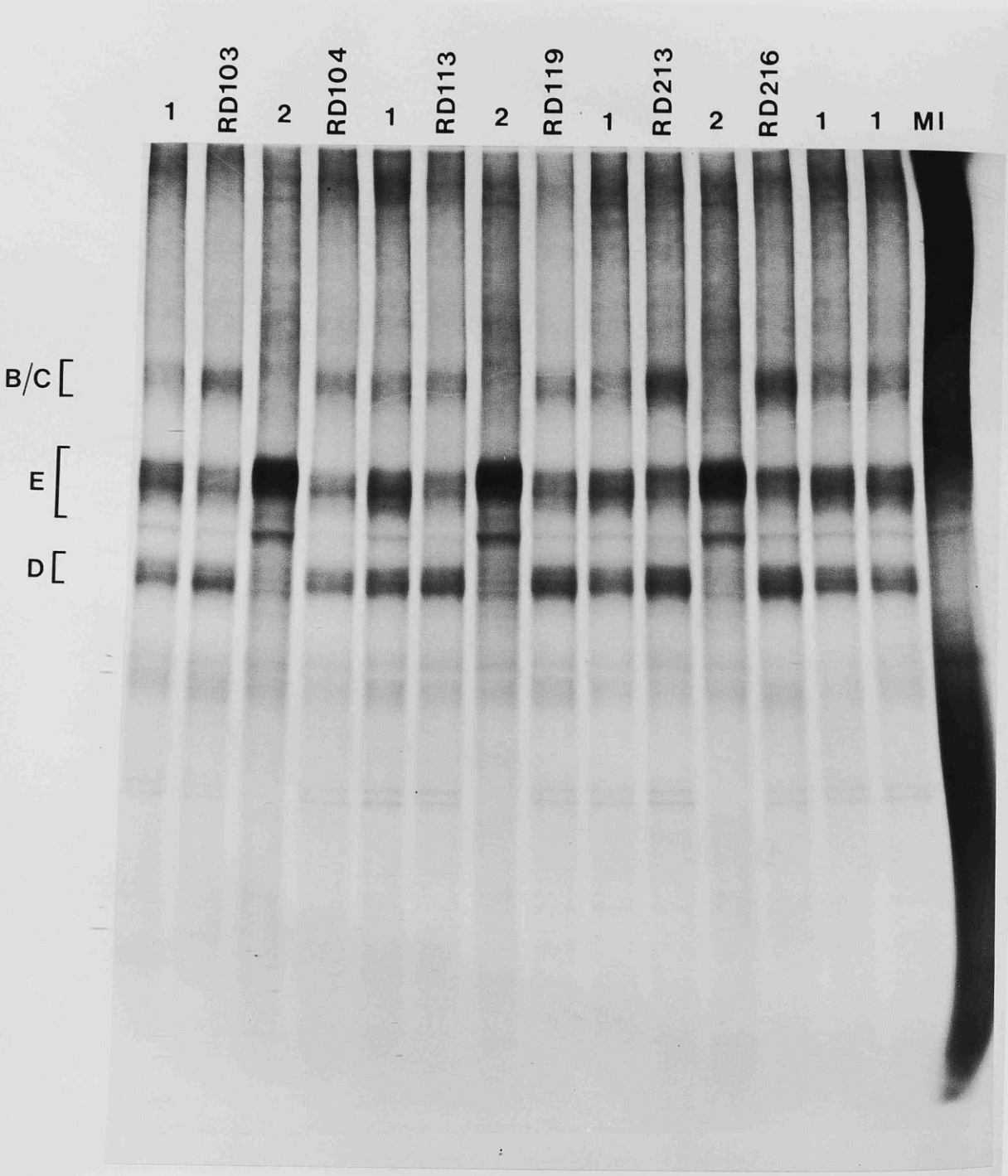

1
RD103
2
RD104
1
RD113
2
RD119
1
RD213
2
RD216
1
1
MI
B/C
E
D

FIGURE 36

Mapping of glycoprotein E. Fluorograph of sulphated polypeptides induced in mock-infected (MI) cells and in cells infected with the intertypic recombinants RD103, RD104, RD113, RD119, RD213, RD216 and the parental viruses HSV-1 strain 17 syn^+ (lanes 1) and HSV-2 strain HG52 (lanes 2). Cells were labelled with ^{35}S-inorganic sulphate from 2-22h after infection.

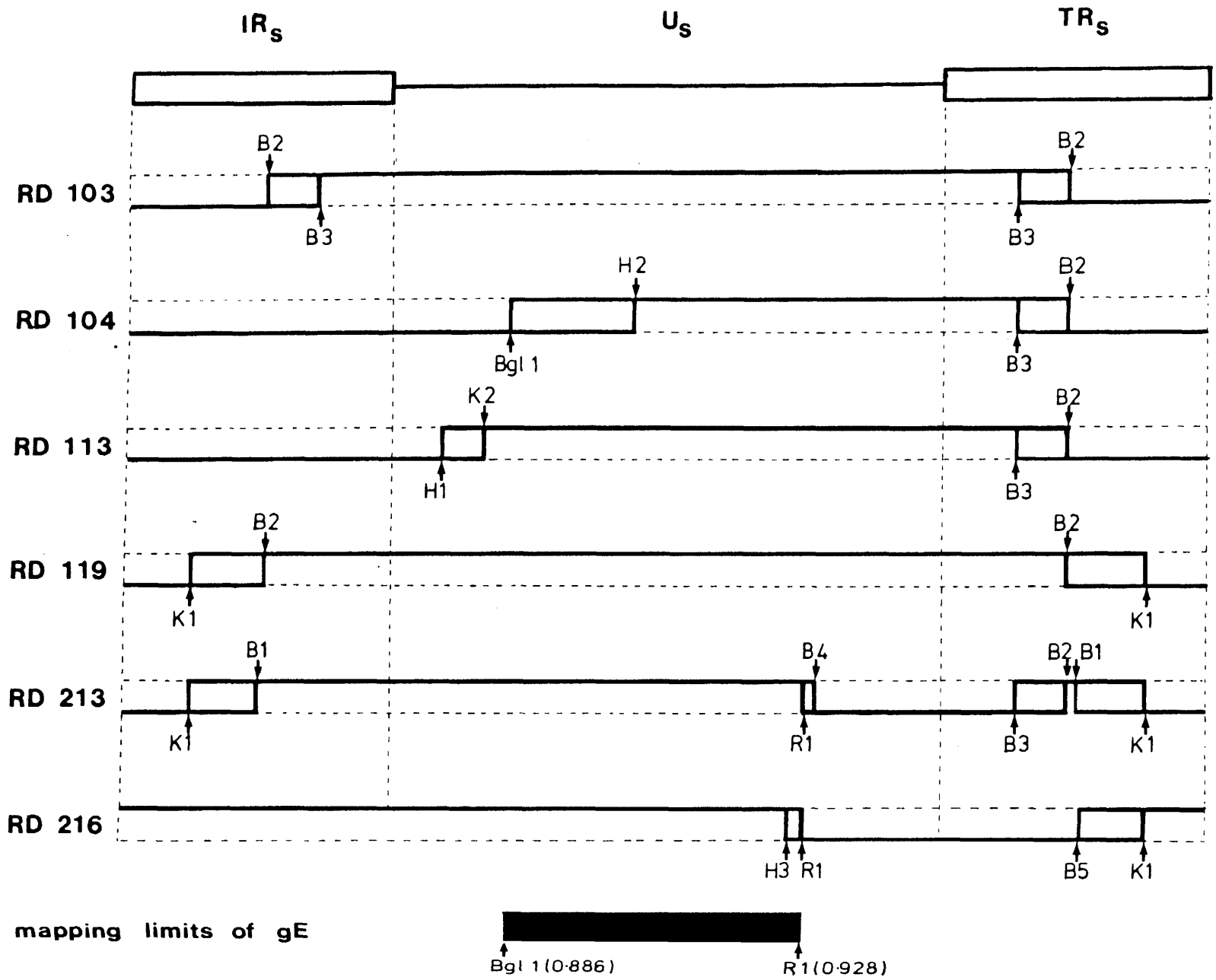

IRS
US
TRS
RD 103
B2
B3
B2
B3
RD 104
H2
Bgl 1
B2
B3
RD 113
K2
H1
B2
B3
RD 119
B2
K1
B2
K1
RD 213
B1
K1
B4
R1
B2 B1
B3
K1
RD 216
H3 R1
B5
K1
mapping limits of gE
Bgl 1(0·886)
R1(0·928)

FIGURE 37

Genome structures of the S region of the six recombinants that were used for the experiment represented in fig. 36, to determine the map location of glycoprotein E. HSV-1 and HSV-2 DNA sequences are indicated by a thickening of the upper or lower, respectively, of the two horizontal dotted lines. Regions of uncertainty of the crossover positions are indicated by the space between thick vertical black lines. The solid black rectangle shows the map position of glycoprotein E. Delimiting restriction sites in HSV-1 DNA (↓) and HSV-2 DNA (↑) are as follows:

B1, BamHI q-m'; B2, BamHI m'-y; B3, BamHI a'-m'; B4, BamHI j-n'; B5, BamHI g'-u; HI HindIII m-l; H2, HindIII n-g; H3, HindIII l-k; KI KpnI r-a; K2, KpnI j-h; BglI, BglII q-l; RI, EcoRI n-o.

Recombinants RD113 and RD119 have HSV-2 sequences in the L segment of the genome extending from co-ordinate 0.00 to 0.10 and from 0.00 to 0.07 respectively.

APPARENT
MW x 10^-3
HSV-1
MI
1
17+ x 11r(1)
2
B x 1 (28-1-1)
1
B x 6(17-1)
2
RE 6
1
RE 4
2
63
35
34
32

FIGURE 38

Mapping of the secreted polypeptides of apparent MW 32,000, 34,000 and 35,000. Fluorograph of polypeptides induced in cells infected with recombinants $17^{+}x11^{r}$ (1), Bx1 (28-1-1), Bx6 (17-1), RE6, RE4 and the parental viruses 17 syn^{+} (HSV-1) and HG52 (HSV-2). Cells were labelled with ^{35}S-inorganic sulphate from 3 to 24h after infection. The symbol (■) indicating an HSV-1 polypeptide or (○) indicating an HSV-2 polypeptide are placed to the left of the polypeptide to which it refers. The numbers 1 or 2 above a lane denotes the HSV-1 or HSV-2 parental virus.

1
RD103
2
RD104
1
RD113
2
RD119
1
RD213
2
RD216
1
1
MI
35
34
32

FIGURE 39

Mapping of the secreted polypeptides of apparent MW 32,000, 34,000 and 35,000. Fluorograph of polypeptides induced in cells infected with recombinants RD103, RD104, RD113, RD119, RD213, RD216 and the parental viruses HSV-1 strain 17 syn^+ (lanes 1) and HSV-2 strain HG52 (lanes 2). Secreted polypeptides are from the growth medium of cells labelled with ^{35}S-inorganic sulphate from 2-20h after infection.

* The HSV-2 induced 63K cannot be mapped safely since it migrates in the region of a M1 protein (figs. 38 and 39).

BglII q-l site (0.886) and on the right-hand side by the HSV-2 EcoRI n-o site (0.935) with all data consistent.

Since the genomic location of the 32K, 34K and 35K proteins is within the limits obtained for gE, the possibility exists that they might be related to gE. They could also be related to gD which was mapped, using intertypic recombinants, to within the region 0.824 to 0.945mu (Marsden et al., 1978; Ruyechan et al., 1978; Halliburton, 1980).*

3.8 Genesis of the 32K, 34K, 35K and the 55K, 57K secreted proteins and the 70K sulphated protein which accumulates in the presence of tunicamycin

3.8.1 Immunoprecipitation with anti-gE monospecific antiserum

Two observations suggested that the 70K polypeptide might be related to gE. First, its mobility on SDS-polyacrylamide gels is very similar to that of pgE (fig. 33). Second, if 70K is a partially processed form of a virus-induced glycoprotein, then the fully mature form of that glycoprotein is likely to be at least as heavily sulphated as 70K. Only gE satisfies this criterion. Accordingly, we tested whether the 70K polypeptide could be immunoprecipitated by an antiserum directed against gE (fig. 40). Cells infected with strain 17 syn$^+$ in the presence or absence of tunicamycin were labelled with either [^{35}S]-methionine or ^{35}S-inorganic sulphate. Extracts were prepared (lanes 1, 2, 7 and 8) and immunoprecipitations performed as described by Lee et al. (1982a). The immune serum, but not control non-immune serum, precipitated gE and pgE from cells infected in the absence of the drug (compare lanes 4 and 6 with lanes 12 and 10 respectively). The 70K polypeptide was specifically precipitated by the anti-gE serum (lanes 3 and 5) but not by the control serum (lanes 11 and 9) demonstrating that it is antigenically related to gE.

That the 32K, 34K and 35K secreted proteins might be antigenically related to gE is also shown in fig. 40. The 32K and 34K

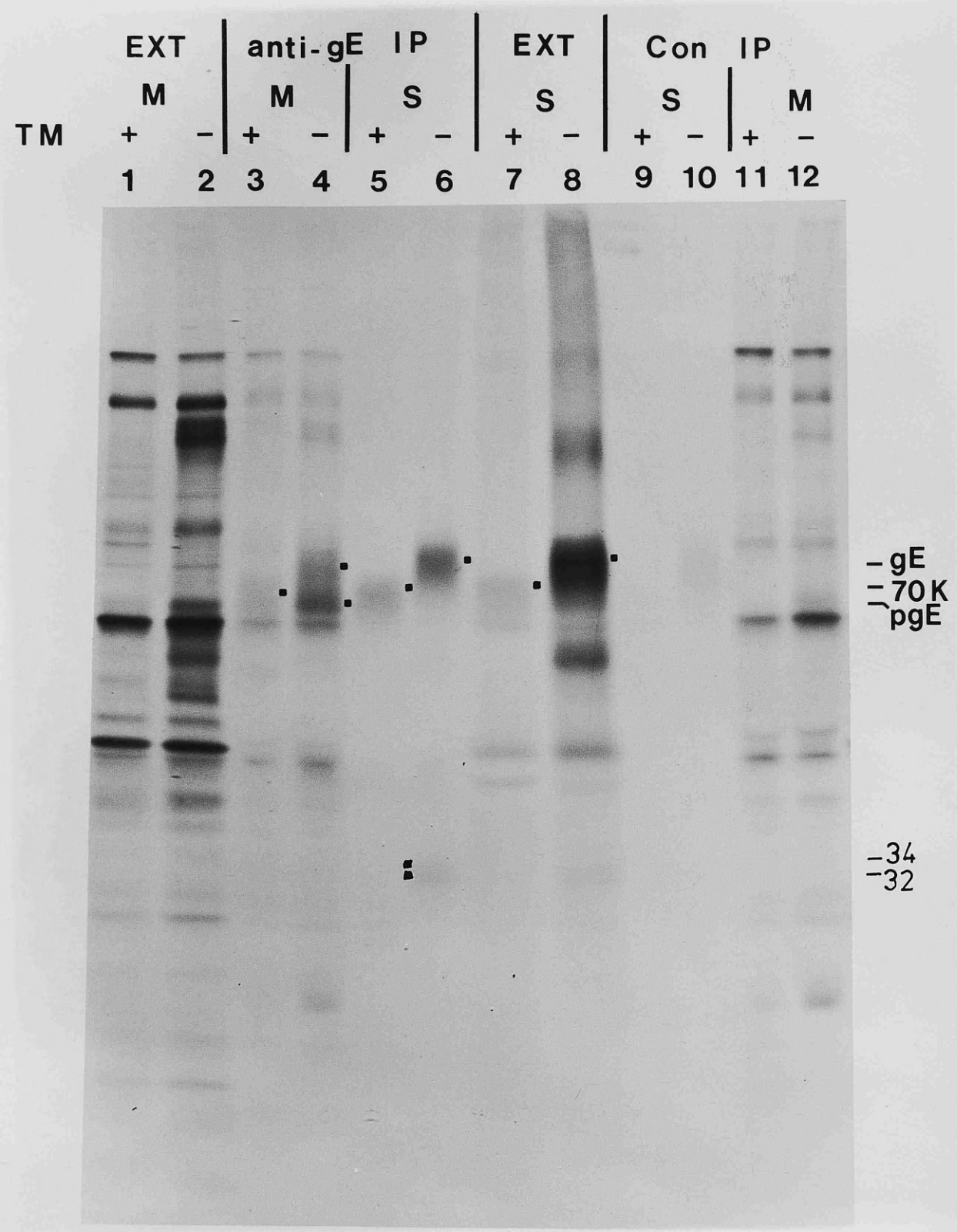

EXT
anti-gE IP
EXT
Con IP
M
M
S
S
S
M
TM + − + − + − + − + − + −
1 2 3 4 5 6 7 8 9 10 11 12
gE
70K
pgE
34
32

FIGURE 40

Antigenic relatedness of gE and the 70K polypeptide which accumulates in the presence of tunicamycin. BHK21 cells were infected with HSV-1 strain 17 syn^+ in the presence (+; lanes 1, 3, 5, 7, 9, 11) or absence (-; lanes 2, 4, 6, 8, 10, 12) of tunicamycin and labelled from 3.5 to 24h after infection with either 5uCi/ml [^{35}S]-methionine (M; lanes 1 to 4, 11, 12) or 500uCi/ml ^{35}S-inorganic sulphate (S; lanes 5-10). Proteins were extracted (EXT) and immunoprecipitated with monospecific rabbit anti-gE serum (Anti-gE IP; lanes 3, 4, 5, 6) or non-immune rabbit serum (Con IP; lanes 9, 10, 11, 12). The positions of gE, pgE, 70K, 34K and 32K are indicated by dots (■) to the right of the appropriate lanes.

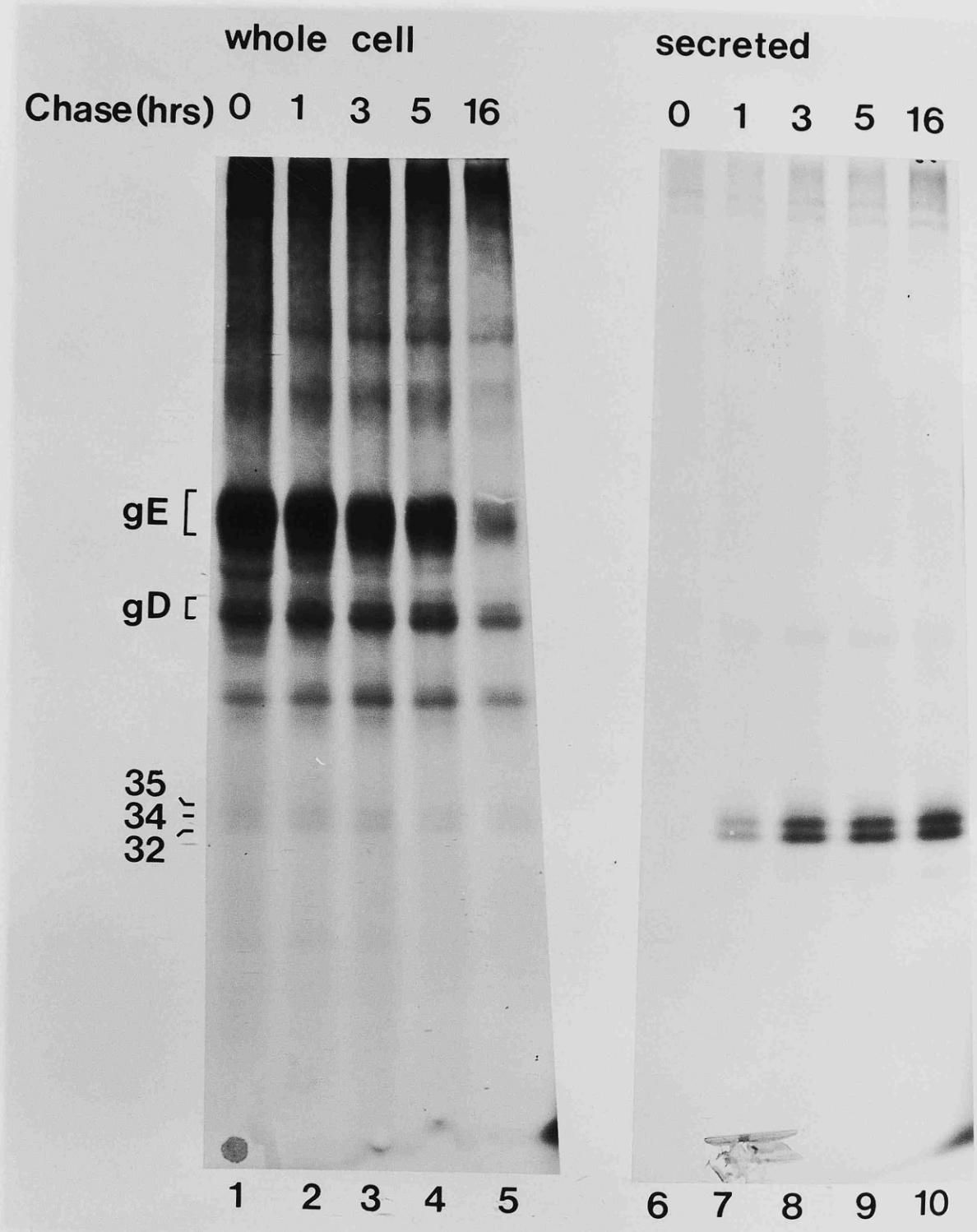

whole cell
secreted
Chase(hrs) 0 1 3 5 16
0 1 3 5 16
gE
gD
35
34
32
1 2 3 4 5
6 7 8 9 10

FIGURE 41

Kinetics of secretion and stability of sulphated polypeptides induced by HSV-1 strain 17 syn^+. Infected cells were labelled with ^{35}S-inorganic sulphate from 5-7h after infection. At the end of this period, the label was removed and cells were then harvested (chase = 0h) or chased for the times indicated (1, 3, 5 or 16h) in medium containing no radioisotope. Secreted polypeptides (lanes 6-10) were obtained for each time point represented in whole infected cells (lanes 1-5).

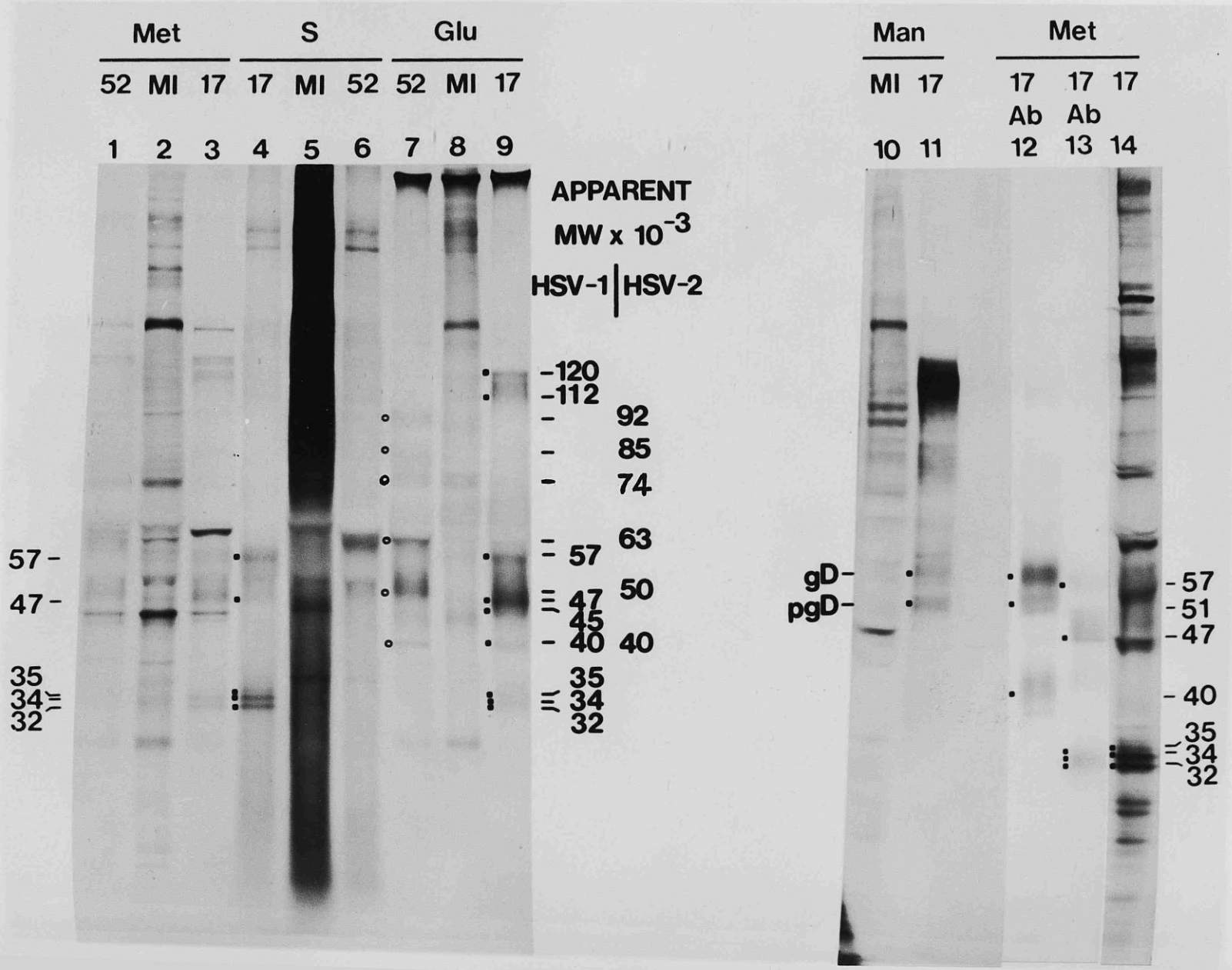

Met
S
Glu
52 MI 17 17 MI 52 52 MI 17
1 2 3 4 5 6 7 8 9
APPARENT
MW x 10-3
HSV-1 | HSV-2
120
112
92
85
74
63
57
50
47
45
40
40
35
35
34
32
57
47
35
34
32
Man
Met
MI 17 17 17 17
Ab Ab
10 11 12 13 14
gD
pgD
57
51
47
40
35
34
32

FIGURE 42

Comparison of polypeptides secreted from infected cells (labelled with [^{35}S]-methionine, ^{35}S-inorganic sulphate and [^{14}C]-glucosamine) with those immunoprecipitated by a monoclonal antibody directed against gD. Polypeptides were labelled with [^{35}S]-methionine (Met, lanes 1 to 3), ^{35}S-inorganic sulphate (S, lanes 4 to 6) or [^{14}C]-glucosamine (Glu, lanes 7 to 9). Polypeptides secreted from mock-infected (MI) cells (lanes 2, 5 and 8) or cells infected with either HSV-1 strain 17 syn^{+} (lanes 3, 4 and 9) or HSV-2 strain HG52 (lanes 1, 6 and 7) were harvested. pgD and gD were identified by the [^{3}H]-mannose-labelled intracellular polypeptides of 17 syn^{+} infected cells (lane 11). Lane 12 shows [^{35}S]-methionine-labelled intracellular polypeptides precipitated by monoclonal antibody number 140. Lane 14 shows [^{35}S]-methionine-labelled polypeptides secreted from cells infected with 17 syn^{+} and lane 13 shows an immune precipitate, by the same antibody, of these polypeptides. Immunoprecipitations were performed by Dr. J.W. Palfreyman.

polypeptides from 17 syn+-infected cells labelled with [^{3}H]-mannose (lane 11). Lane 13 shows the polypeptides precipitated by this antibody from the total [^{35}S]-methionine-labelled polypeptides secreted from infected cells (lane 14). The 32K, 34K and 35K polypeptides were specifically precipitated apparently demonstrating that they share a common antigenic determinant with gD.

This result is clearly not compatible with experiments (fig. 40) showing precipitation of the secreted proteins by anti-gE antibodies. The results which follow investigate this anomaly and demonstrate unambiguously that the 32K, 34K and 35K proteins are related to gE. This earlier result arose most likely by monoclonal antibody No. 140 being a mixture of two specificities, one against gD and the other against the secreted proteins. Unfortunately, it is not possible to test this hypothesis as the antibody producing cell line is no longer viable and the stock of antibody is depleted.

3.8.4 Purification of gD, gE and the secreted proteins 32K, 34K, 35K, and 55K, 57K by immunoaffinity chromatography using monoclonal antibodies directed against gD, gE and the 32K, 34K and 35K proteins

To determine the origin of the 32K, 34K and 35K secreted proteins, it was decided to first purify them and glycoproteins D and E and analyse the purified proteins by tryptic peptide mapping. The proteins were first purified by immunoaffinity chromatography using monoclonal antibodies directed against glycoproteins D, E and the 32K, 34K and 35K proteins. The antibodies were covalently linked to sepharose and immunoaffinity columns prepared as described (Section 2.6.2). Fig. 43 shows an autoradiograph of proteins purified by immunoaffinity chromatography (lanes 2, 3, 4 and 6) from extracts (lanes 1 and 5) of HSV-1-infected cells labelled with [^{35}S]-methionine from 5h to 12h after infection. The anti-gB monoclonal antibody was used as a control to

WC EXT
gD-2001/1
gE-3104
gB-2975
Sup EXT
SP-3114/109
gB
mD/pgB
gE
pgE
57K/gD
55K
pgD
SP
63K
actin
43K
1
2
3
4
5
6

FIGURE 43

Purification of HSV-1 strain 17 syn$^+$-induced polypeptides by immunoaffinity chromatography. Infected BHK cells were labelled from 5-12h after infection with [^{35}S]-methionine. Extracts were prepared from whole infected cells (WC EXT) or from the supernatant of these infected cells (Sup EXT). About $3x10^6$ cell equivalents of the WC EXT and about $1x10^7$ cell equivalents of the Sup EXT were passed through the immunoaffinity columns containing monoclonal antibodies (MABs) bound to sepharose. The MABs used were directed against: gD-MAB 2001/1, lane 2; gE-MAB 3104, lane 3; gB-MAB 2957, lane 4 and the 32,000, 34,000, 35,000 secreted proteins -MAB 3114/109, lane 6. Bound proteins were eluted with 3M Na SCN pH7.8, then concentrated 20-30 fold using PEG 6000. Specifically bound proteins are indicated on the left-hand side of the autoradiograph. Non-specifically trapped proteins are indicated on the right-hand side of the autoradiograph: relatively major contaminants are identified by a MW [e.g. 63,000 (63K)] or named (e.g. actin), relatively minor contaminants are indicated by an arrow (←).

identify proteins non-specifically trapped on the columns: these are indicated on the right-hand side of the figure and include several high MW proteins (arrowed), probably of host origin, the host actin protein and the viral 63K and 43K proteins. The anti-gD monoclonal antibody recognises gD, pgD and a high MW protein thought to be a multimer of gD (mD) which co-migrates with pgB (lane 2). The anti-gE monoclonal antibody recognises gE and pgE (lane 3) while the anti-32K, 34K and 35K monoclonal antibody recognises the 32K, 34K and 35K proteins, a 57K protein which co-migrates with gD and a 55K protein (lane 6).

3.8.5 Tryptic peptide maps of gD, gE, 55K, 57K and the 32K, 34K and 35K proteins

To obtain single proteins for tryptic peptide fingerprinting the proteins purified by immunoaffinity chromatography were separated by SDS-PAGE using a 10% single concentration preparative gel. The gels were dried and autoradiographs prepared, then using the autoradiographs as templates, strips of acrylamide containing the proteins of interest were excised, eluted from the acrylamide and tryptic peptide maps prepared as described in Section 2.10.2. In addition to gD, gE and the secreted proteins, it was decided to prepare maps of the putative multimer of gD (mD) and the 55K, 57K proteins recognised by the anti-32K, 34K and 35K antibodies. Fig. 44 shows the tryptic peptide maps of these proteins and the pattern obtained by mixtures of tryptic peptides of gE and the 32K, 34K and 35K proteins and also of gD and the 32K, 34K and 35K proteins. It can be seen that gE, the 32K, 34K and 35K and the 55K, 57K proteins, gave similar tryptic peptide maps and were distinct from gD. The relatedness of gE, 32K, 34K and 35K secreted proteins and the 55K, 57K secreted proteins was unambiguously established by examination of the mixtures of tryptic peptides, which shows the spots seen in the maps of secreted proteins co-migrate with the spots seen in the gE map, but not

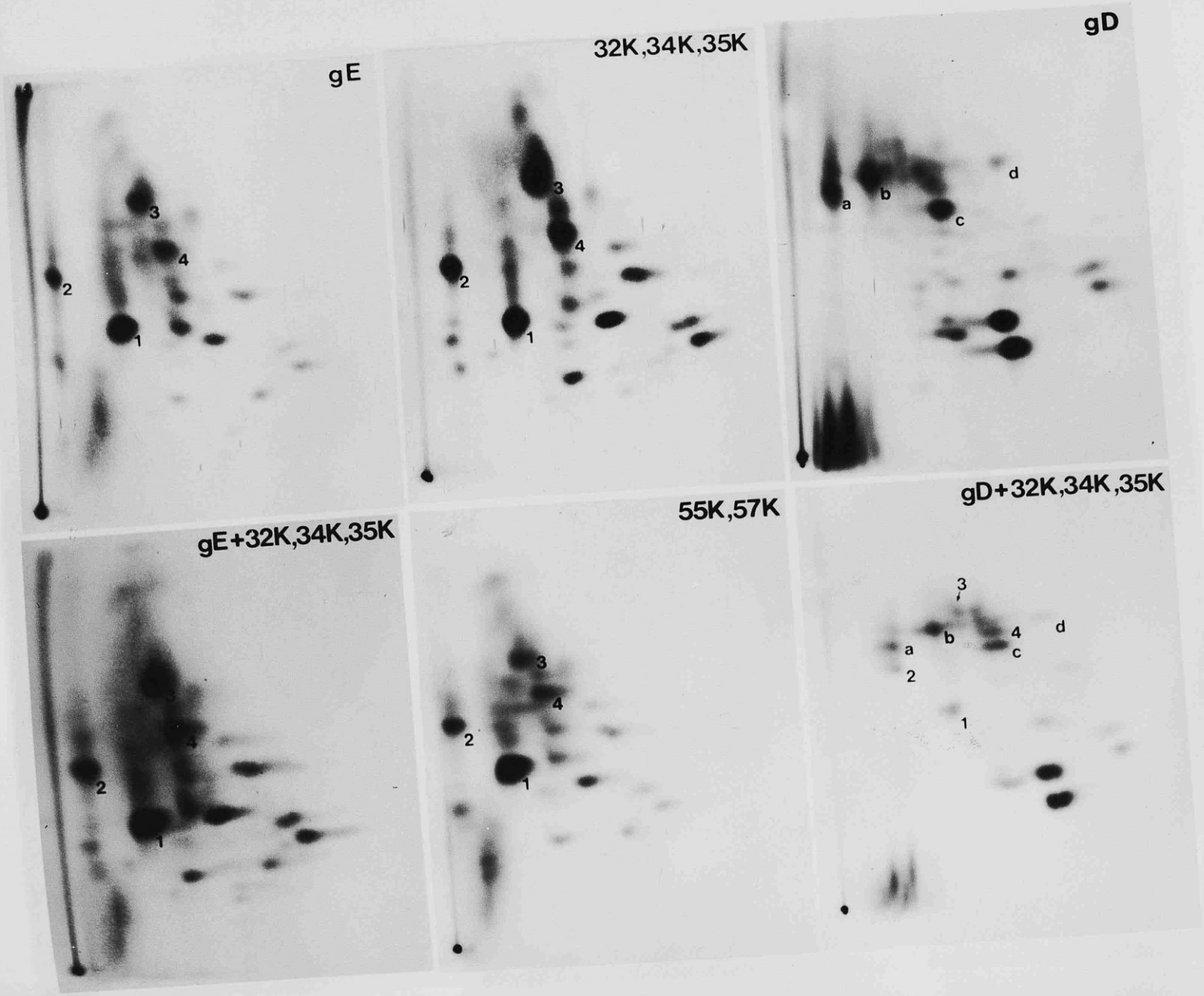

gE
32K,34K,35K
gD
gE+32K,34K,35K
55K,57K
gD+32K,34K,35K

FIGURE 44

Fluorographs showing 2-dimensional tryptic peptide maps of immunoaffinity purified proteins from HSV-1 strain 17 syn$^+$-infected cells; top row, first panel - gE, second panel - secreted proteins 32K, 34K, 35K, third panel - gD: bottom row, first panel - a mix of the gE and 32K, 34K, 35K peptides, second panel - secreted proteins 55K, 57K, third panel - a mix of the gD and 32K, 34K, 35K peptides. Purified extracts shown in fig. 43 were separated on 10% single concentration SDS-polyacrylamide gels and the following proteins excised: (a) gD and mD from the MAB 2001/1 immunoaffinity column, (b) gE from the MAB 3104 immunoaffinity column, (c) the secreted proteins 32K, 34K, 35K and 55K, 57K from the MAB 3114/109 immunoaffinity column. Tryptic peptides were prepared for each protein as described in the Methods. The tryptic peptides were applied to thin layers of cellulose, the point of application (origin) is shown by the black dot at the bottom left of each panel, and peptides were separated first by electrophoresis (vertical direction of the fluorograph) then by ascending chromatography (horizontal direction of the fluorograph). Some tryptic peptides have been designated with a number or a letter to facilitate comparison.

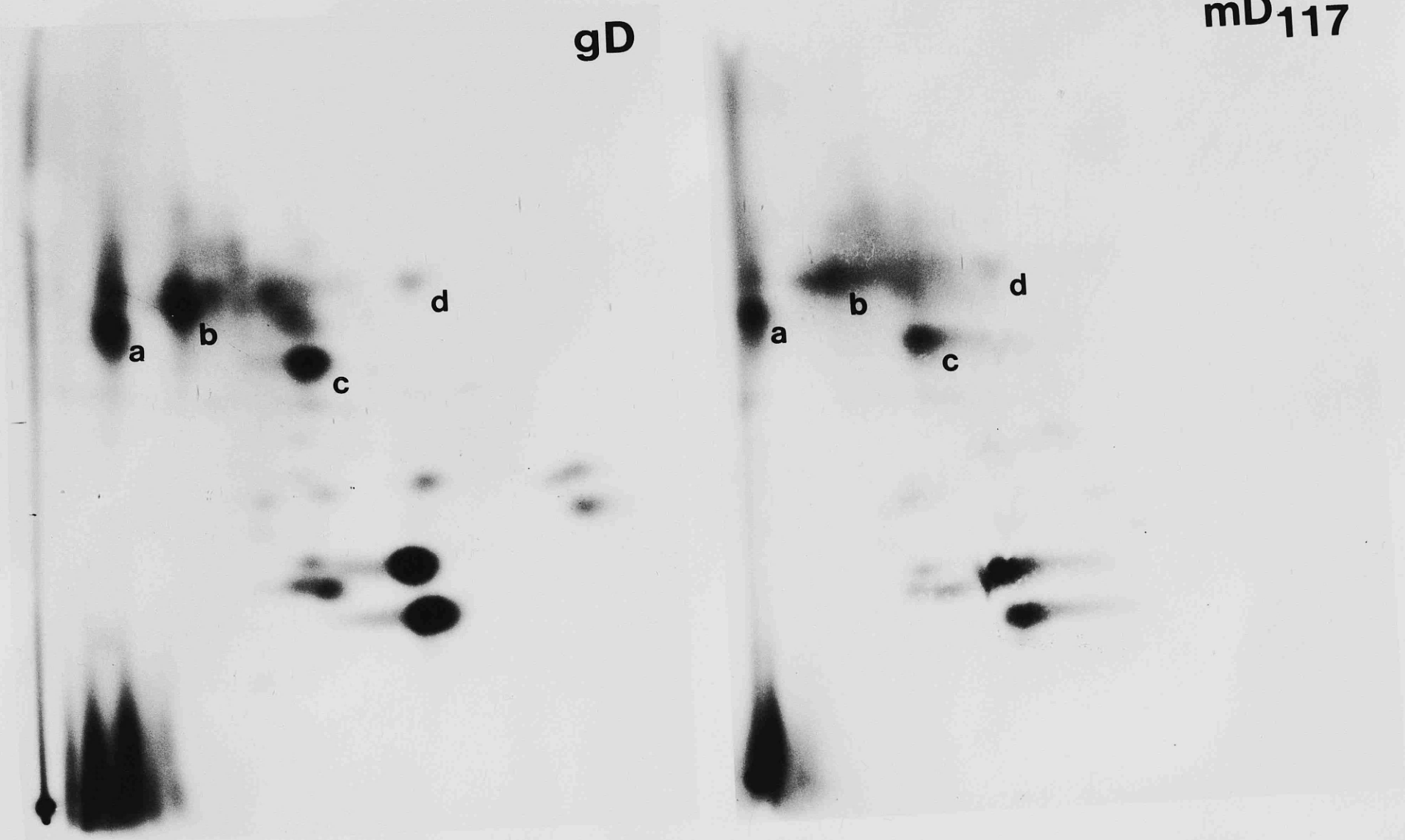

gD
a
b
c
d
mD117
a
b
c
d

FIGURE 45

Fluorograph showing 2-dimensional tryptic peptide maps which demonstrate the antigenic relatedness of mD (apparent MW 117K) and gD. A purified extract, from the anti-gD MAB, 2001/1 immunoaffinity column (see fig. 43) was separated on a 10% single concentration SDS-polyacrylamide gel and the following proteins excised (a) gD, (b) mD. Tryptic peptides were prepared for each protein as described in the Methods and applied to thin layers of cellulose and first separated by electrophoresis (vertical direction of the fluorograph), then by ascending chromatography (horizontal direction of the fluorograph). Some tryptic peptides have been designated with a letter to facilitate comparison.

with the spots seen in the gD map. The tryptic peptide map produced by the high MW protein recognised by the anti-gD monoclonal antibody gave a similar pattern to the gD pattern (fig. 45). It can be concluded that the 32K, 34K and 35K proteins are encoded by the gene which encodes gE. The 55K, 57K proteins (which migrate at the same apparent MW as gD) are also encoded by the gE gene.

3.8.6 Tryptic peptide maps of individual secreted proteins, 32K, 34K and 35K

Although the apparent MW of gE is twice that of each of the 32K, 34K and 35K secreted proteins, the tryptic peptide maps of gE contains no spots additional to those seen in the tryptic peptide maps of 32K, 34K and 35K. This observation raised the possibility that at least two of the 32K, 34K and 35K proteins are encoded by different regions of the gene encoding gE. To test this hypothesis, the 32K, 34K and 35K proteins were first purified by immunoaffinity chromatography using the monoclonal antibody, 3114/109 column. The 32K, 34K and the 35K were individually cut out of 10% SDS gels and tryptic peptides prepared. Fig. 46 shows autoradiographs of the tryptic peptide maps of 32K, 34K and 35K and a mix of the 34K and 35K tryptic peptides. The patterns produced by the 34K and the 35K look similar. This is substantiated by examining the mix of the 34K and 35K peptides. Although this sample has not run into the cellulose sheet as far as the individual 34K and 35K maps, it does show that the tryptic peptides for both the 34K and 35K co-migrate. Furthermore, the map of the mixture of 34K and 35K tryptic peptides looks similar to the map of the 32K tryptic peptides. It is concluded that each of the three secreted proteins 32K, 34K and 35K are all encoded, at least, in part by a common region of the gE gene. This still leaves unresolved the question as to why there are as many tryptic peptides in the maps of the secreted proteins as in the map of gE. The question is

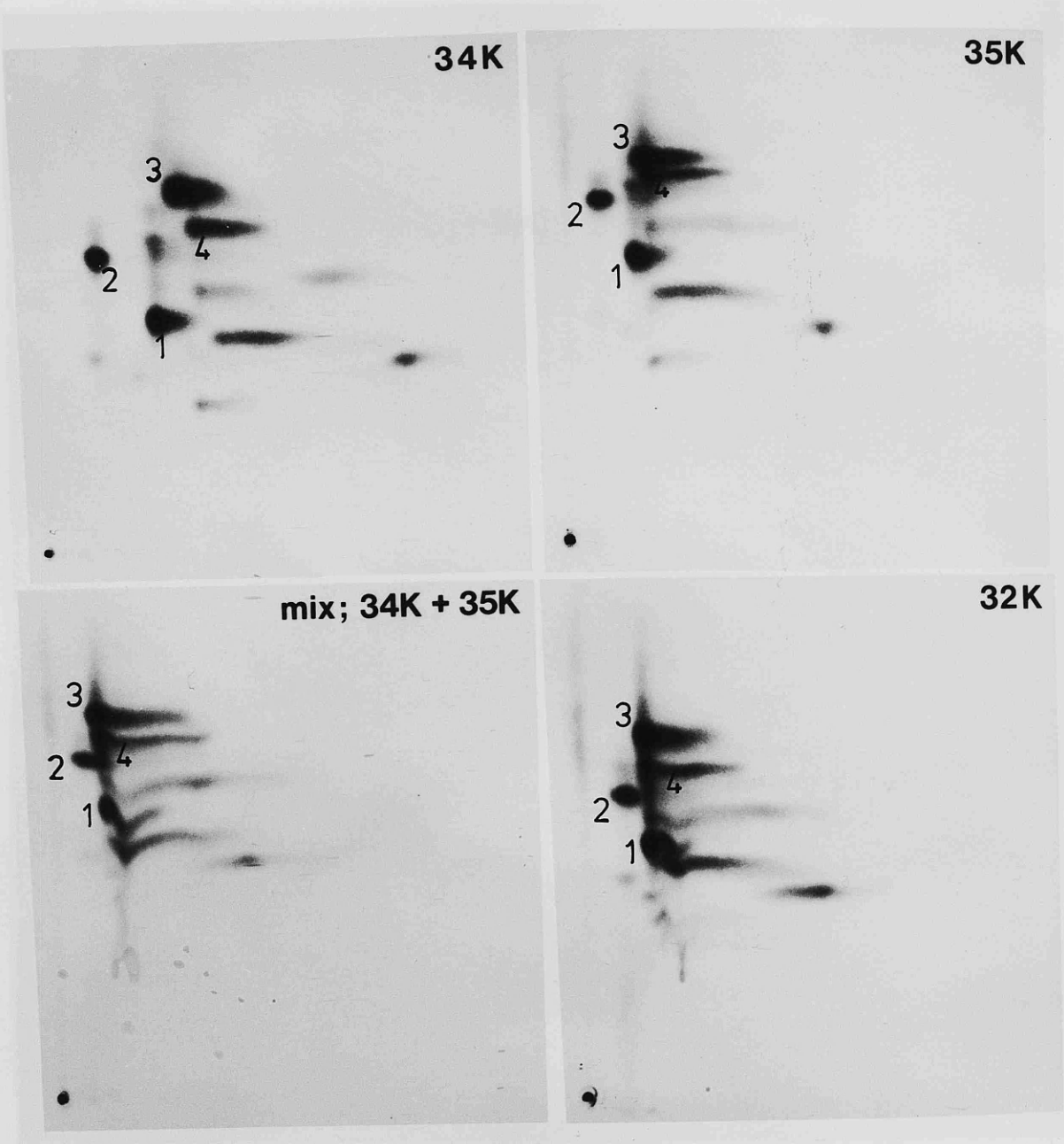

34K
3
4
2
1
35K
3
4
2
1
mix; 34K + 35K
3
2
4
1
32K
3
4
2
1

FIGURE 46

Fluorograph showing 2-dimensional tryptic peptide maps of the individual components of the secreted proteins: top left, secreted protein of apparent MW 34,000 (34K); top right, secreted protein 35K; bottom left, a mix of the 34K and 35K; bottom right, secreted protein 32K. The secreted proteins were purified by immunoaffinity chromatography from HSV-1 strain 17 syn^+-infected cells labelled from 2-22h after infection with [^{35}S]-methionine, as shown in fig. 43. The purified secreted proteins were separated on a 10% single concentration SDS-polyacrylamide gel. The 32K, the 34K and the 35K secreted proteins were excised separately from the gel. Tryptic peptides were prepared from each protein and applied to thin layers of cellulose and firstly separated by electrophoresis (vertical direction of fluorograph) then by ascending chromatography (horizontal direction of chromatography). Several tryptic peptides have been designated with a number to facilitate comparison.

discussed in Section 4.6.

3.8.7 Generation of the secreted proteins 32K, 34K and 35K by a serum component

Baucke and Spear (1979) identified gE by virtue of its ability to bind the Fc-region of IgG. Since the 32K, 34K and 35K proteins are encoded by the gE gene, it was of interest to test whether they also possessed this property. To do this, Fc-affinity columns consisting of rabbit anti-bovine serum albumin (BSA) antibodies bound to BSA which was itself covalently bound to sepharose 4B were prepared as described by Baucke and Spear (1979). Control columns lacked rabbit antibody (see Section 2.7). Equal volumes of either a whole infected cell extract labelled with ^{35}S-inorganic sulphate or a supernatant extract from the infected cells were applied to the affinity or control columns and eluted as described. Those proteins which did not bind to the columns were also collected. The results are presented in fig. 47 in which lanes 6'-10' are heavier exposures of lanes 6-10. In agreement with the results of the experiment shown in fig. 31, the major sulphated glycoprotein in cells infected with 17 syn+ was more strongly bound by the Fc-affinity column (lane 2) than the control column (lane 3). The 32k, 34K and 35K secreted proteins are identified in the whole cell extract (lane 1) and in the supernatant (lanes 6 and 6'). They do not bind to the Fc-affinity columns (lanes 2, 7 and 7') or the control columns (lanes 3, 8 and 8') but proteins which co-migrate with them appear in greatly increased quantities in the flow through material of the whole cell extract from the Fc-affinity column (lane 5), compared with the flow through material from the control column (lane 4). From this experiment it can be concluded that the 32K, 34K and 35K secreted proteins do not bind to the Fc domain of IgG. The results also suggest they can be generated in an Fc-affinity column by cleavage of gE. As expected, the amount of 32K, 34K and 35K in the

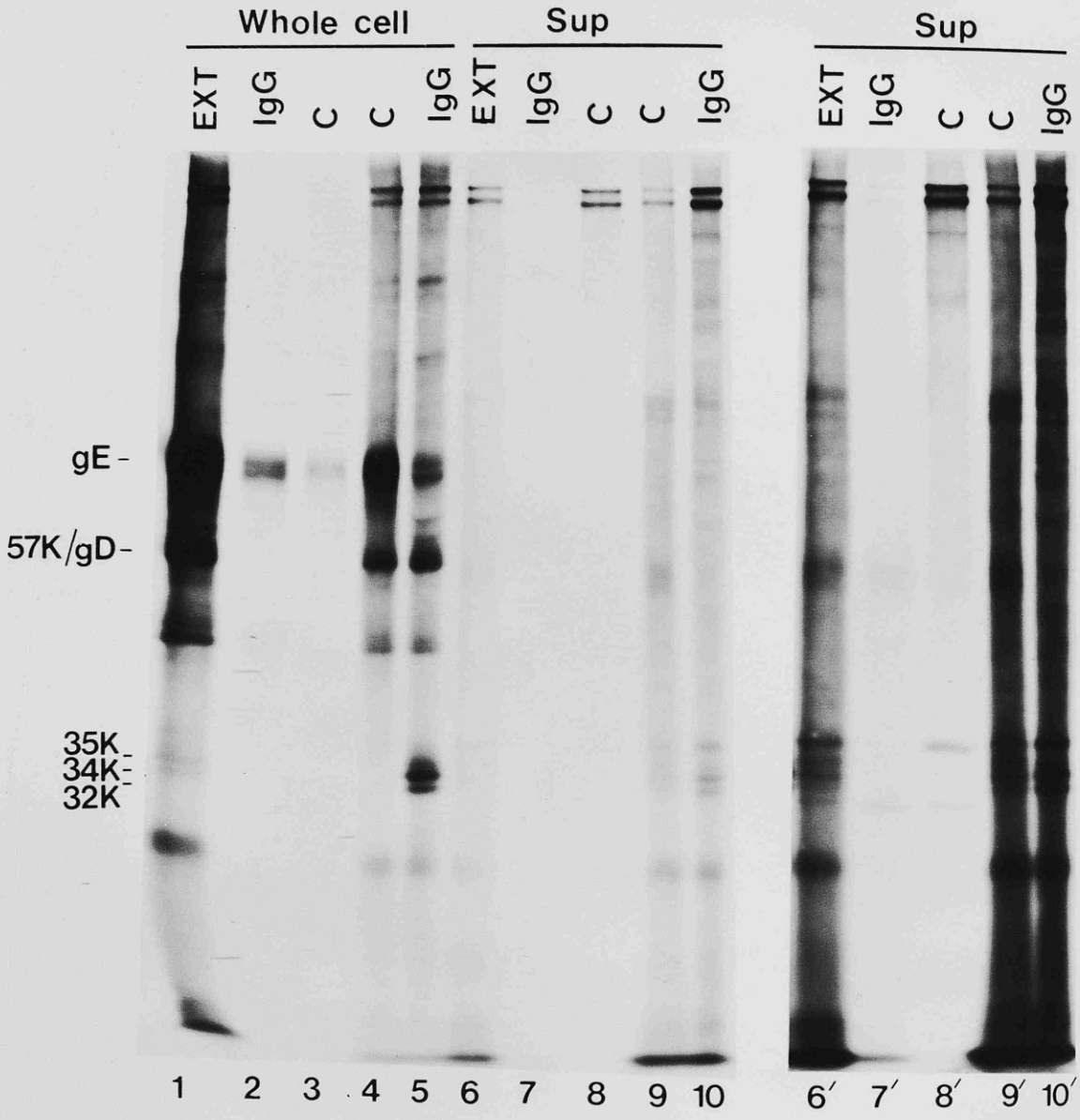

Whole cell
Sup
Sup
EXT
IgG
C
C
IgG
EXT
IgG
C
C
IgG
EXT
IgG
C
C
IgG
gE
57K/gD
35K
34K
32K
1
2
3
4
5
6
7
8
9
10
6′
7′
8′
9′
10′

FIGURE 47

Generation of the secreted proteins 32K, 34K, 35K from an extract of HSV-1-infected cells on an Fc-affinity column. Extracts (EXT) of infected cells (whole cell) labelled with ^{35}S-inorganic sulphate from 5-12h after infection (lane 1) and the supernatant of a 2h, 35000g centrifugation (Sup) of the infected cells (lanes 6, 6') were applied to Fc-affinity columns (IgG), which contained antibodies directed against bovine serum albumin (BSA) bound to BSA-sepharose, and control columns which contained only BSA-sepharose. That part of the sample which came through the column without binding (lanes 4, 5, 9, 10) was collected. After extensive washing, proteins bound to either the Fc-affinity column (lanes 2, 7) or control column (lanes 3, 8) were eluted with 3M Na SCN as described by Baucke and Spear (1979). Lanes 6'-10' are from an autoradiograph which was exposed for approximately seven times longer than that from which lanes 1-10 were taken.

supernatant fraction from both Fc-affinity column and control column is similar (compare lanes 10' with 9') since the supernatant contains no gE to be cleaved.

Evidence that a serum component may be responsible for the cleavage of gE-1 to generate 32K, 34K and 35K is shown in fig. 48. gE-1 was purified by immunoaffinity chromatography as described in Methods, Section 2.8.1 from BHK cells infected with HSV-1 strain 17 syn+ and labelled with ^{35}S-inorganic sulphate from 5h-12h after infection (lane 2). The purified gE-1 was then incubated for 5h at 37°C in the absence of serum (lane 3) or the presence of 2% calf serum (lane 4), 2% human serum (lane 5) or 2% non-immune rabbit serum (lane 6). Lane 1 serves to indicate the mobility of the 32K, 34K and 35K polypeptides. The experiment shows that in the absence of serum, gE-1 is stable at 37°C (compare lanes 2 and 3), but in the presence of calf and rabbit serum but not human serum polypeptides which co-migrate with the 32K, 34K and 35K polypeptides accumulate. The amount of 32K, 34K and 35K polypeptides detected after the addition of human serum to purified gE-1, varied from experiment to experiment, but it was consistently less than that produced by calf or rabbit serum. When purified immunoglobulin was added in three similar experiments instead of whole serum, no 32K, 34K or 35K polypeptides could be detected (data not shown). These results suggest that a serum protein(s), but not immunoglobulin only, may be involved in proteolytic cleavage of gE-1.

To investigate whether secretion of the 32K, 34K and 35K polypeptides from infected cell monolayers was dependent on the presence of serum in the growth medium, serum-starved BHK cells were infected with HSV-1 strain 17 syn+ and pulse-labelled with ^{35}S-inorganic sulphate from 5h-6h after infection in the absence or presence of calf or human serum. Infected cells labelled in the absence of serum were either

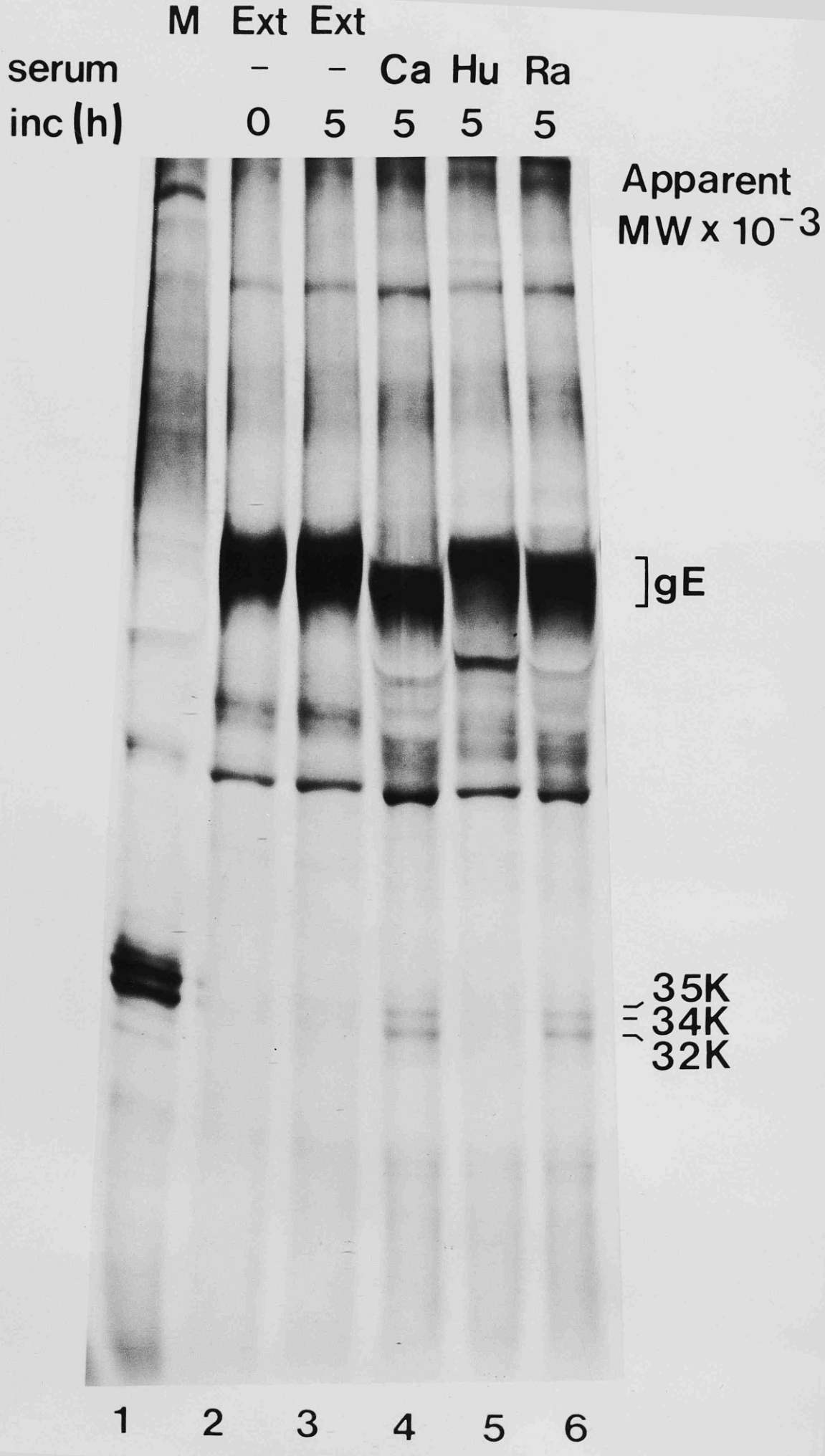

M
Ext
Ext
serum
–
–
Ca
Hu
Ra
inc (h)
0
5
5
5
5
Apparent
MW x 10⁻³
]gE
35K
34K
32K
1
2
3
4
5
6

FIGURE 48

Generation of the 32K, 34K and 35K secreted proteins from purified gE by incubation with serum. An extract of BHK cells infected with HSV-1 strain 17 syn^+ and labelled from 5-12h after infection with ^{35}S-inorganic sulphate was passed through an anti-gE immunoaffinity column. Bound gE was eluted (EXT) with 3M NaSCN and incubated (inc) at 37°C for the hours (h) indicated, either in the absence of serum (-; lane 3) or the presence of 2% calf serum (Ca; lane 4), 2% human serum (Hu; lane 5) or 2% rabbit serum (Ra; lane 6). Lane 2 shows purified gE before incubation. Lane 1 contains 32K, 34K, 35K polypeptides purified by immunoaffinity chromatography from the supernatant of BHK cells infected with HSV-1 strain 17 syn^+ labelled with [^{35}S]-methionine from 2-24h.

harvested immediately or incubated for a further 5h in PBS containing no serum. Infected cells labelled in the presence of serum were either harvested immediately or incubated for a further 5h in PBS containing 2% serum. The results are presented in fig. 49. In the absence of serum, no 32K, 34K, 35K polypeptides were secreted either during the pulse (lane 5) or the subsequent chase (lane 6). In the presence of calf serum, the 32K, 34K and 35K polypeptides are secreted in minor amounts during the pulse (lane 3) and in large amounts during the subsequent chase (lane 4). Small amounts of the 55K, 57K proteins were also secreted during the chase. In the presence of human serum, no 32K, 34K, 35K polypeptides were detectably secreted during the pulse although small amounts of the 55K, 57K polypeptides were detected (lane 7). During the chase, large amounts of the 55K, 57K, but only small amounts of the 32K, 34K, 35K, polypeptides were secreted (lane 8). Other proteins secreted in the presence of serum are of unknown origin.

It can be concluded from this series of experiments that in HSV-1-infected cells, in tissue culture, gE-1 can be post-translationally modified to yield the 32K, 34K, 35K, 55K and 57K polypeptides by a mechanism which involves at least one serum protein. The serum protein(s) cannot be immunoglobulin only and the relative yield of particular proteins depends on the source of the serum.

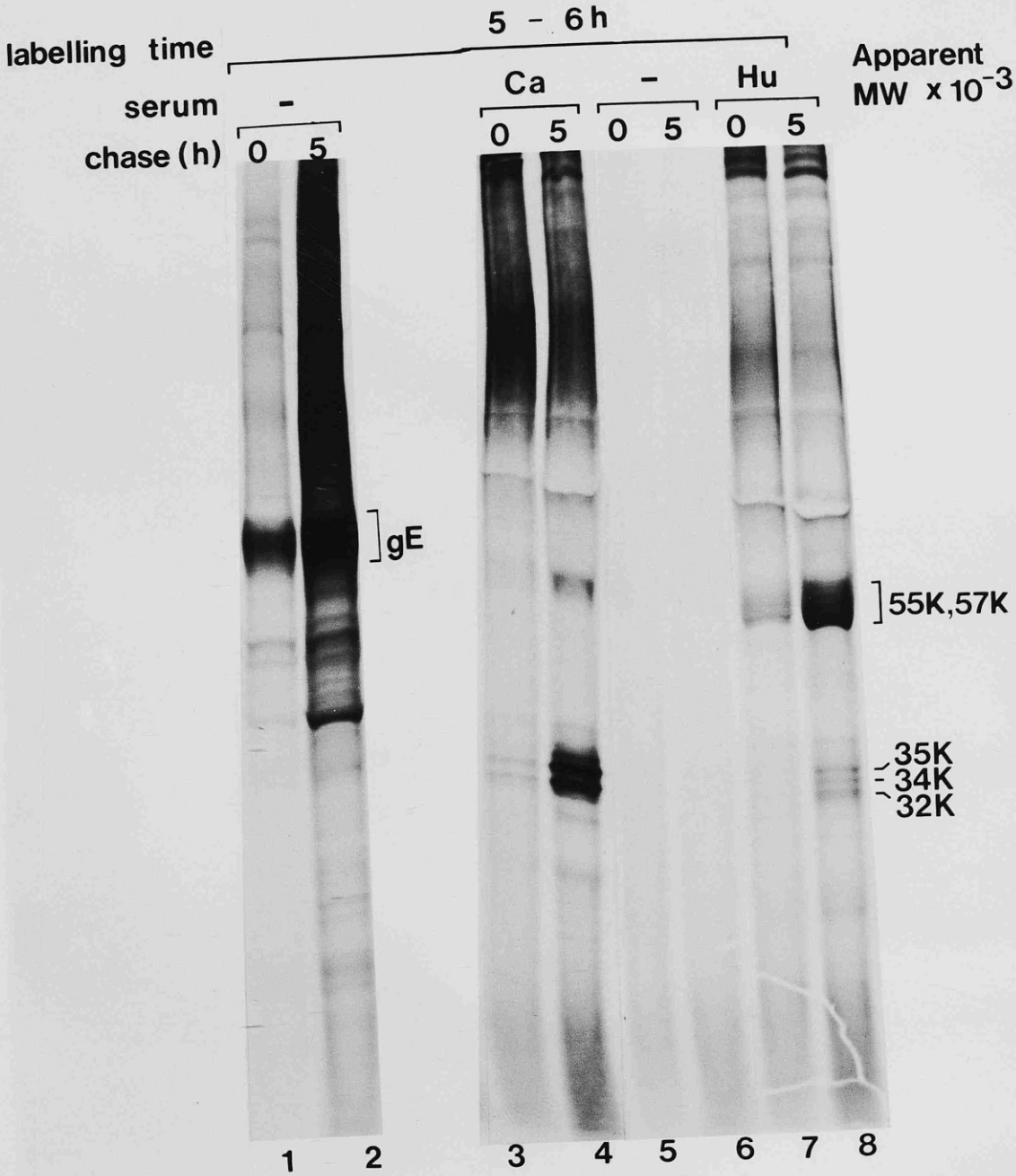

labelling time
5 - 6h
serum
-
Ca
-
Hu
chase (h)
0
5
0
5
0
5
Apparent MW x 10-3
gE
55K,57K
35K
34K
32K
1
2
3
4
5
6
7
8

FIGURE 49

Serum dependence of secretion of 32K, 34K, 35K, 55K and 57K from infected cells. Serum-starved BHK cells infected with HSV-1 strain 17 syn^+ were pulse-labelled with ^{35}S-inorganic sulphate from 5-6h after infection, in the absence (-) or presence (Ca, Hu) of serum as indicated. The label was then removed and cells were either harvested immediately (lane 1; chase = 0h) or incubated for a further 5h (lane 2; chase = 5h) in PBS containing no serum. Lanes 3 to 8 are from a different gel and represent proteins secreted from cells into the growth medium of these infected cells. The MWs of the secreted proteins are indicated on the right-hand side of the figure.

SECTION B: IDENTIFICATION OF A NEW HSV-2-INDUCED GLYCOPROTEIN g92K

3.9 Carbohydrate and inorganic sulphate composition of g92K

g92K had been previously identified on the basis of its incorporation of [^{14}C]-glucosamine (Marsden et al., 1978). To investigate further the nature of the glycoprotein, the incorporation of other radioactively labelled precursors was examined and the results are shown in fig. 27. Under the conditions used, the 92000 (92K)-dalton glycoprotein labelled heavily with glucosamine, only moderately with mannose and poorly, if at all, with inorganic sulphate (lanes 10, 3 and 9). These combined labelling characteristics distinguished g92K from gD and gE. In fig. 27, g92K appears as a doublet (lane 10). There is no readily identifiable HSV-1 equivalent for this glycoprotein (lane 12) although a possible candidate is discussed in Section 4.8.

3.10 Immunoprecipitation with monoclonal antibodies AP1 and LP5

Work in the laboratory of A.T. Minson showed that these two antibodies precipitated a glycoprotein which co-migrated with gB on 7.5% gels cross-linked with N,N'-methylenebisacrylamide. However, work by J.W. Palfreyman (Marsden et al., 1984) established that the target antigen of the monoclonal antibodies mapped in the short region of the HSV genome and was clearly different from the locus of gB. At this stage, it was considered possible that the target antigen of AP1 and LP5 and g92K were one and the same protein. To investigate this possibility the mobility of the protein precipitated by AP1 and LP5 on the 5%-12.5% SDS-PAGE gels used to identify g92K was examined, and the physical map location of g92K was narrowed using additional HSV-1 x HSV-2 intertypic recombinants.

BHK cells were infected with HSV-2 strain HG52 and labelled with

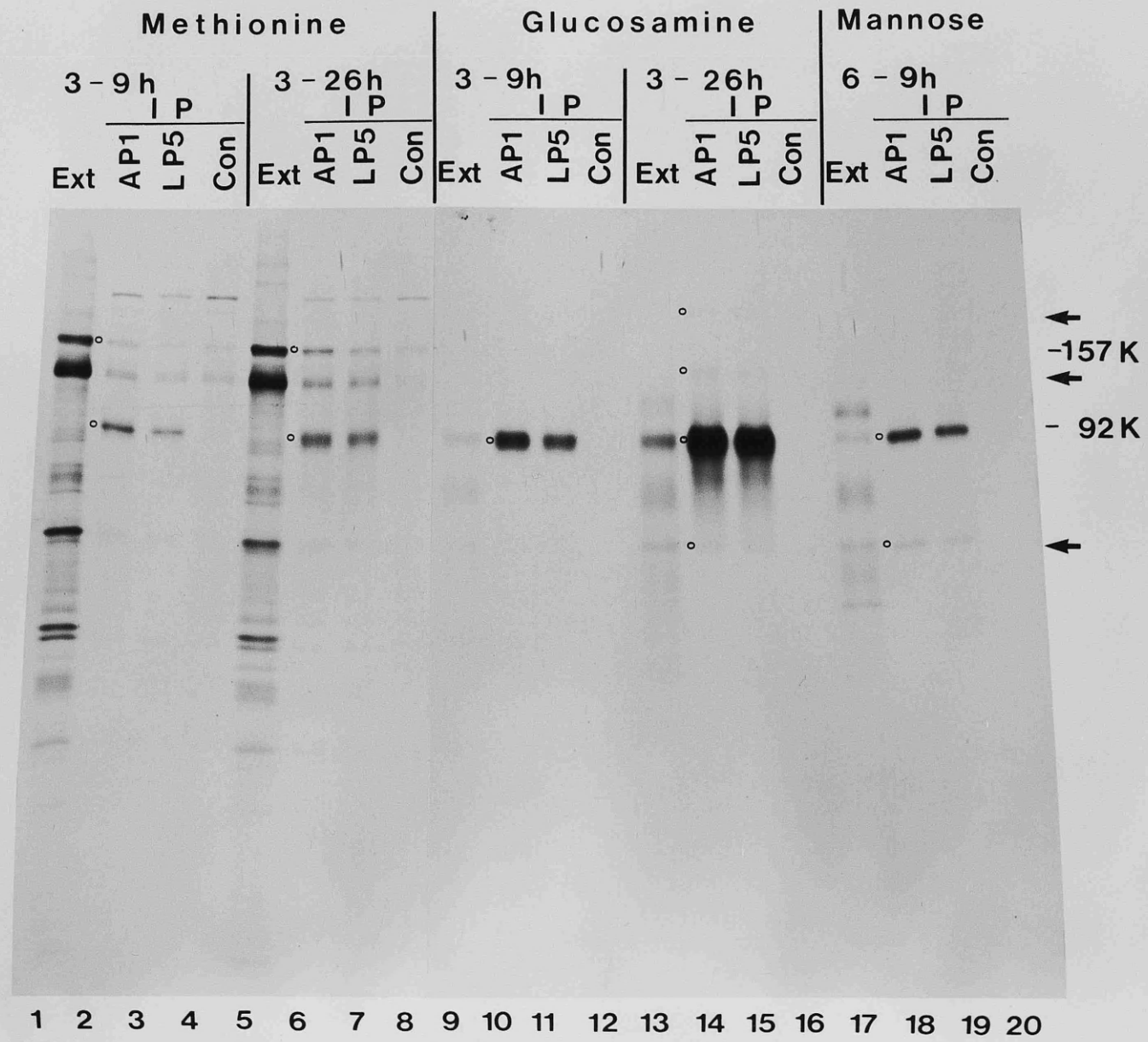

Methionine
Glucosamine
Mannose
3 - 9 h
3 - 26h
3 - 9h
3 - 26h
6 - 9h
I P
Ext
AP1
LP5
Con
-157 K
- 92 K
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIGURE 50

Immune precipitation of the g92K protein from HSV-2 strain HG52 infected cells labelled with [^{35}S]-methionine, [^{14}C]-glucosamine and [^{3}H]-mannose. The infected cells were labelled for the times indicated and an extract was made (Ext) which was used for immunoprecipitation (IP) with two anti-g92K monoclonal antibodies (AP1 and LP5) and a control ascites fluid (Con). Symbols: ◄ minor bands precipitated by AP1 and LP5; o , HSV-2 glycoproteins.

[^{35}S]-methionine, [^{14}C]-glucosamine and [^{3}H]-mannose for the times indicated in fig. 50. Extracts were prepared (lanes 1, 5, 9, 13 and 17) and immunoprecipitations performed as described by McLean et al. (1982).

Both AP1 and LP5 specifically immunoprecipitated a [^{35}S]-methionine-labelled HSV-2 g92K polypeptide (fig. 50) (lanes 2, 3, 6 and 7) which was present in only trace amounts in the extract (lanes 1 and 5). Several other proteins (e.g. 157K, the major capsid protein) were precipitated by these antisera, but not specifically since they were also precipitated by the control ascites fluid (lanes 4 and 8). When [^{14}C]-glucosamine was employed as label, AP1 and LP5 specifically precipitated g92K (lanes 10, 11, 14 and 15). They similarly immunoprecipitated a [^{3}H]-mannose-labelled polypeptide with an apparent MW of 92K from HSV-2-infected cells (lanes 18 and 19), demonstrating that g92K contains both glucosamine and mannose. Three other bands were sometimes precipitated by AP1 and LP5 from glucosamine-labelled (upper two arrows, lanes 14 and 15) or glucosamine- and mannose-labelled polypeptides (lower arrow, lanes 14, 15, 18 and 19). These polypeptides are present in the precipitates in much lower amounts than g92K, and their relationship to g92K remains to be established. Comparison of the 3h to 9h results with the 3h to 26h results (compare lane 10 with lane 14 and lane 11 with lane 15) suggested that g92K is synthesised in increased amounts late in infection.

3.11 Physically mapping g92K by SDS-PAGE

The polypeptide profiles of eleven HSV-1 x HSV-2 intertypic recombinants were studied: the presence of g92K and the serotype of gD are presented for all recombinants in Table 14. Figs. 51, 53 and 54 show relevant portions of fluorographs of the gels on which [^{14}C]-glucosamine-labelled polypeptides induced by Bx6 (17-1), Bx1 (28-1-1), RD104, RD113, RD213, RE4, RE6 and R12-1, the parental 17 syn+ and HG52 strains, and

TABLE 14

Mapping of g92K and gD by SDS-PAGE

Recombinant	Presence of g92K (HSV-2)[a]	gD serotype[b]
Bx6 (17-1)	+	2[c]
Bx1 (28-1-1)	+	2[c]
RD104	-	1
RD113	-	1
RD213	-	1
RE4	-	1
RE6	-	1
R12-1	-	-[d]
$17^{+}x11^{r}$	+	2
RH6	-	1
RS5	-	1

a Serotype determined from [14C]-glucosamine-labelled profile

b Serotype determined from both [14C]-glucosamine-labelled and [3H]-mannose-labelled profile

c It was not possible to tell the serotype of the protein from fig. 51 but results of other experiments showed these to be HSV-2

d The mobility was faster than that of both type 1 and type 2

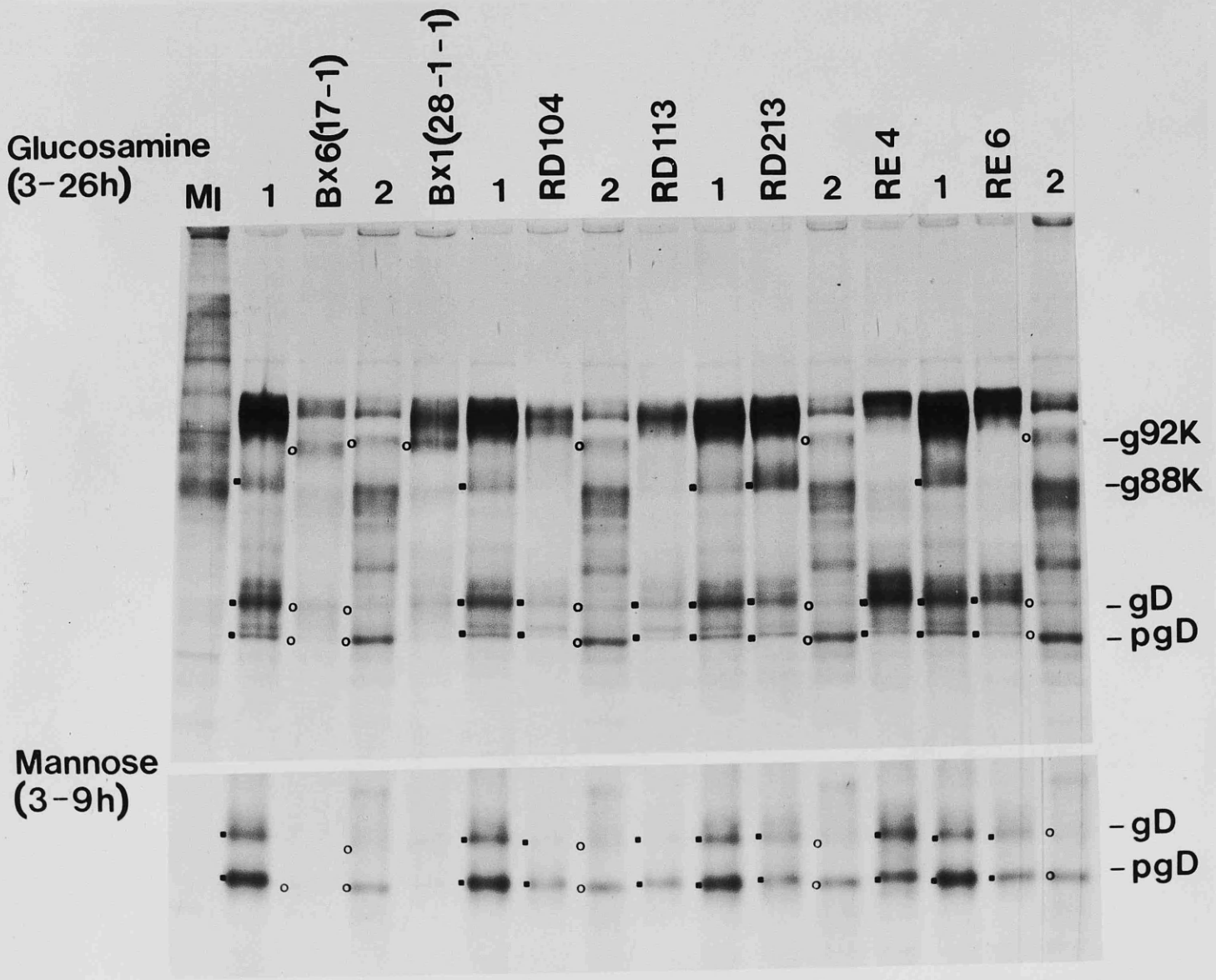

Glucosamine
(3-26h)
MI
1
Bx6(17-1)
2
Bx1(28-1-1)
1
RD104
2
RD113
1
RD213
2
RE 4
1
RE 6
2
-g92K
-g88K
- gD
- pgD
Mannose
(3-9h)
- gD
- pgD

FIGURE 51

Mapping the g92K. Fluorograph of polypeptides labelled with [^{14}C]-glucosamine (upper panel) and [^{3}H]-mannose (lower panel) in mock-infected (MI) cells and in cells infected with Bx6 (17-1), Bx1 (28-1-1) RD104, RD113, RD213, RE4, RE6 and the parental viruses HSV-1 strain 17 syn^{+} (lanes 1) and HSV-2 strain HG52 (lanes 2). The fluorograph of [^{14}C]-glucosamine-labelled polypeptides has been trimmed to show only those polypeptides of MW greater than about 40,000, whereas the fluorograph of [^{3}H]-mannose-labelled polypeptides has been trimmed to show only those polypeptides of MW between 40,000 and 65,000. Symbols: ■, HSV-1 glycoproteins; O, HSV-2 glycoproteins.

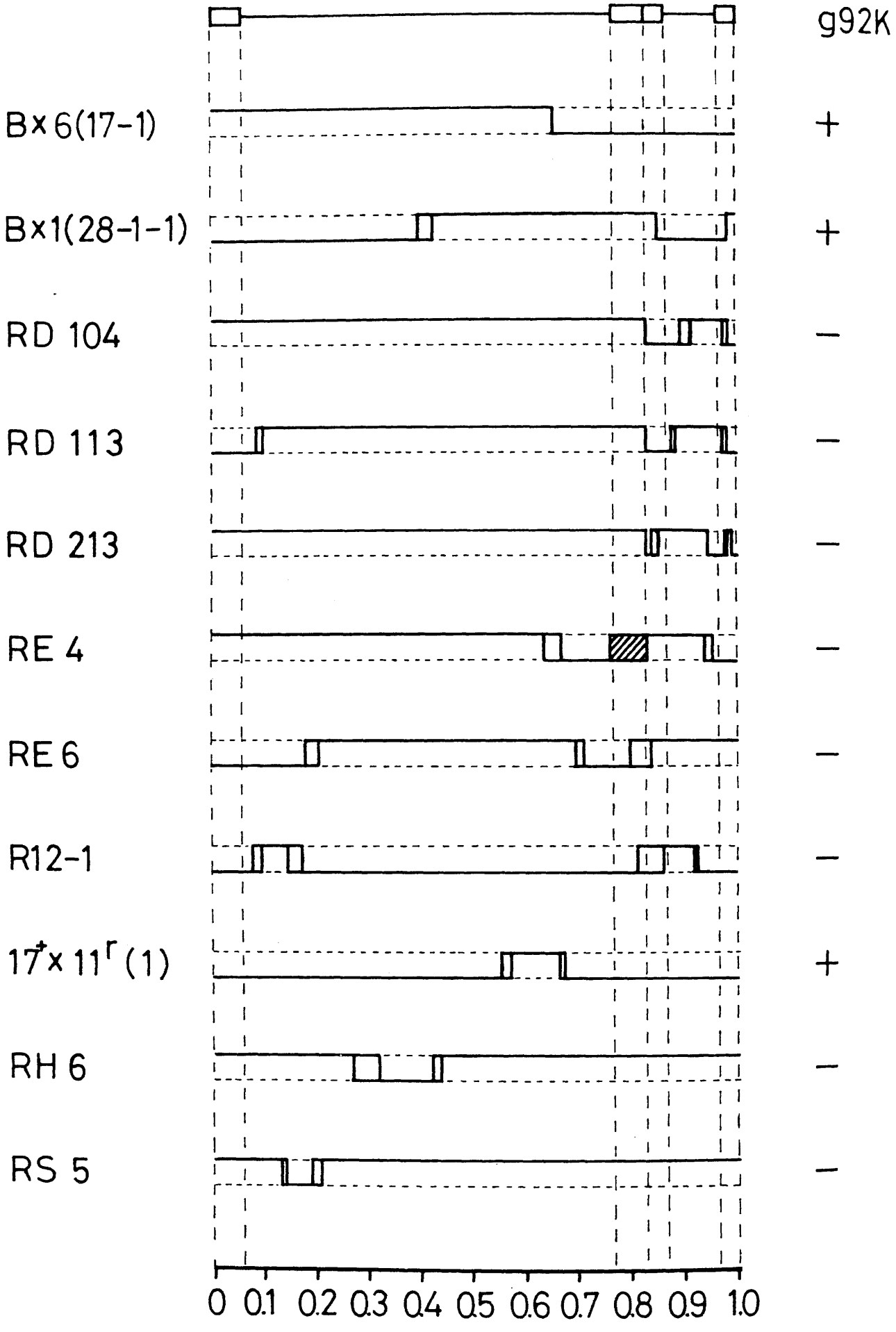

g92K
Bx6(17-1)
+
Bx1(28-1-1)
+
RD 104
−
RD 113
−
RD 213
−
RE 4
−
RE 6
−
R12-1
−
17⁺x 11ʳ (1)
+
RH 6
−
RS 5
−
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

FIGURE 52

Summary of the genome structures of the eleven intertypic recombinants used to map g92K. The genome arrangement of HSV DNA is illustrated at the top of the figure, showing the long and short repeat sequences and the long and short unique regions. Vertical dotted lines correspond to the ends of the long and short repeat sequences. Those sequences of the recombinant derived from the type 1 and type 2 parent are represented by a thick continuous line superimposed on the upper (HSV-1) and lower (HSV-2), respectively of the two horizontal dotted lines. Crossover regions are indicated by one or two vertical lines between the thick continuous horizontal lines. The distance between two vertical lines indicates the remaining region of uncertainty for that crossover event. Where the uncertainty is small, the crossover appears as a single vertical line. The units at the bottom are expressed as a fraction of the genome length. Although RE4 is fixed in the I_S configuration, it is drawn in the prototype configuration, the hatched box represents deleted DNA sequences. There is a transposition of HSV-2-DNA sequences in RE4 from 0.26-0.28mu into the position of the deleted sequences (Davison and Wilkie, 1983). The right of the figure shows for each recombinant whether it induces (+) or does not induce (-) g92K.

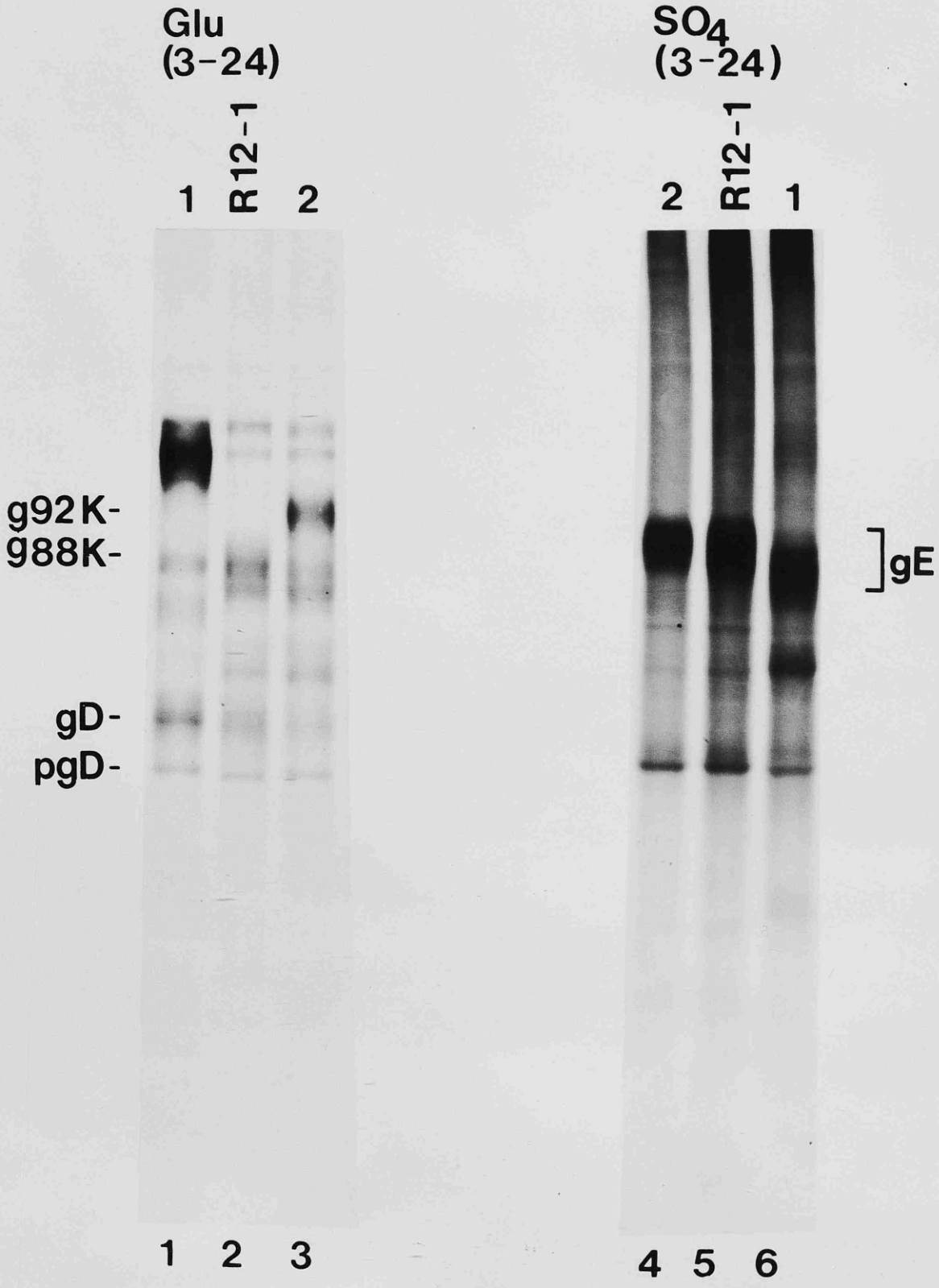

Glu
(3-24)
1
R12-1
2
g92K-
g88K-
gD-
pgD-
1 2 3
SO4
(3-24)
2
R12-1
1
gE
4 5 6

FIGURE 53

Presence of g92K and serotype of glycoproteins D and E in the intertypic recombinant R12-1. Fluorograph of polypeptides labelled with [^{14}C]-glucosamine (lanes 1-3) or ^{35}S-inorganic sulphate (lanes 4-6) in cells infected with R12-1 and the parental viruses HSV-1 strain 17 syn^{+} (lanes designated 1) and HSV-2 strain HG52 (lanes designated 2). Two gels were used, the ^{35}S-inorganic sulphate-labelled polypeptides migrated a little further than those labelled with [^{14}C]-glucosamine.

MI cells have been separated. It is noted that in fig. 51, the mobility of g92K induced by the two positive recombinants varied from that of g92K induced by HG52, and probably reflects various degrees of processing. Correlation of the data presented in Table 14 with the genome structures of the recombinants (fig. 52) gives a map position for g92K that is delimited on the left by RD104 the HSV-2 BglII q-1 site in U_S (map co-ordinate 0.892) and on the right by R12-1, the HSV-2 BamHI c'-d' site in U_S (map co-ordinates 0.924). All data were individually consistent with this location.

Of particular significance was the serotype of gE induced by recombinant R12-1. The serotype of gE could readily be seen with inorganic sulphate as label (fig. 53). R12-1 did not induce g92K and did induce gE-2. This result strongly suggests that g92K and gE are encoded at least in part by different regions of the genome. The serotype of pgD could be seen with glucosamine as label (figs. 51 and 53) and more clearly with mannose as label (fig. 51). R12-1 induced a pgD with mobility different from that of either type 1 or type 2, although it is noted that this mobility difference was not apparent in the mature form of the glycoprotein (gD). This result provides weakly supportive evidence that gD is also encoded, at least in part, by a different region of the genome from that encoding g92K.

3.12 Mapping and identification of g92K in the glycoproteins secreted from HSV-2 infected cells

To further characterise g92K, it was investigated whether it is secreted from infected cells. A polypeptide which labels heavily with [^{14}C]-glucosamine and with the mobility of g92K was secreted from cells infected with 17+x11r, Bx1 (28-1-1) and Bx6 (17-1), but not from cells infected with RE6, RH6 or RS5 (fig. 54). Correlation of these data with the genome structures (fig. 52) gave a map position in the short region

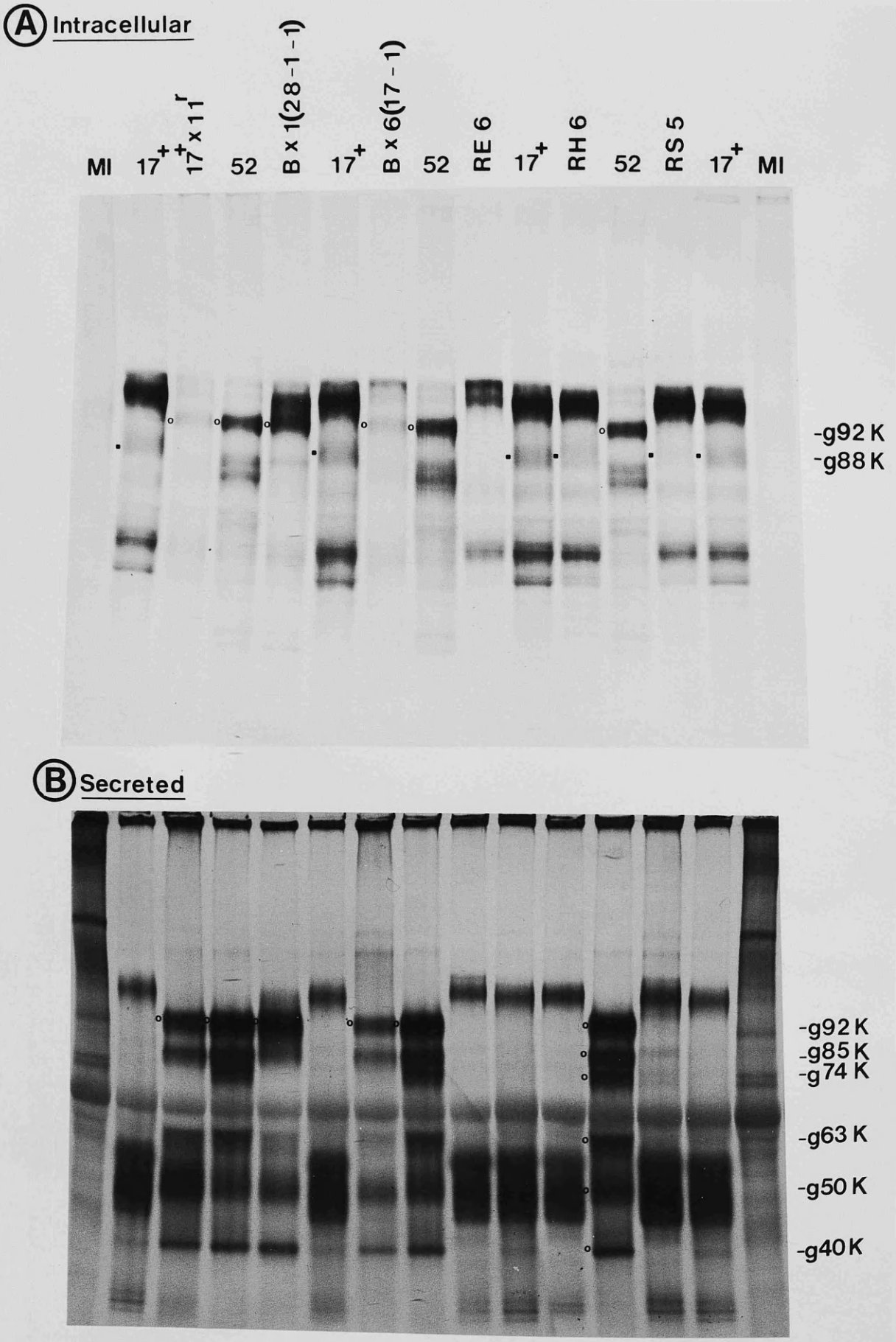

A Intracellular
MI
17+
17+ x 11r
52
B x 1(28-1-1)
17+
B x 6(17-1)
52
RE 6
17+
RH 6
52
RS 5
17+
MI
-g92 K
-g88 K
B Secreted
-g92 K
-g85 K
-g74 K
-g63 K
-g50 K
-g40 K

FIGURE 54

Mapping of g92K secreted proteins. Fluorograph of polypeptides induced in cells infected with $17^{+}x11^{r}$, Bx1 (28-1-1), Bx6 (17-1), RE6, RS5 and the parental viruses 17 syn^{+} (HSV-1) (lanes 17^{+}) and HG52 (HSV-2) (lanes 52) and present either intracellularly (panel A) or secreted from the cell (panel B). Cells were labelled with [^{14}C]-glucosamine from 2-26h after infection. All HSV-2 secreted glycoproteins have been indicated on the most right-hand '52' lane of panel B to aid the comparison in Table 9.

Symbols: ■, HSV-1 glycoproteins; O, HSV-2 glycoproteins.

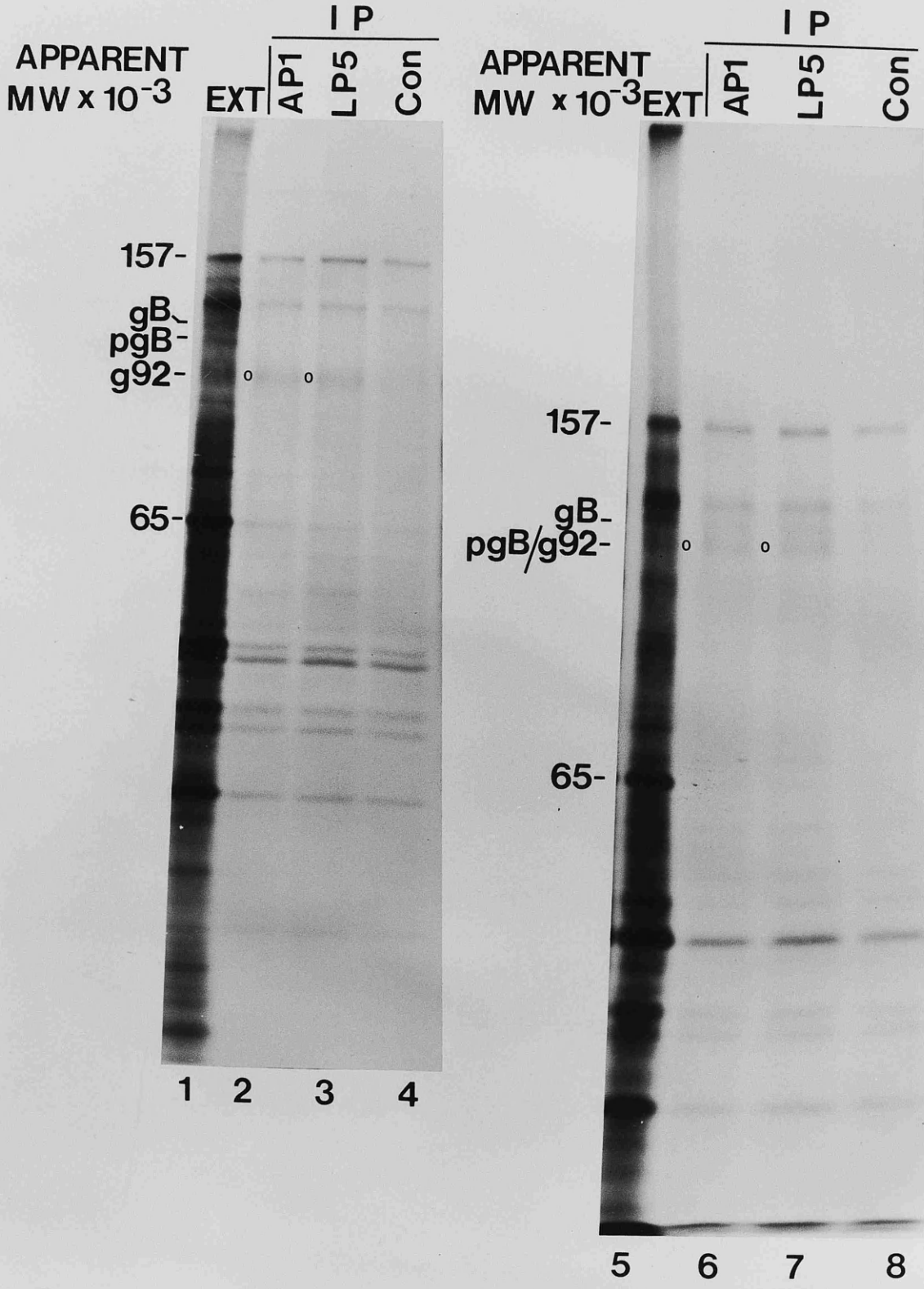

APPARENT
MW x 10-3
EXT
I P
AP1
LP5
Con
157-
gB-
pgB-
g92-
65-
1 2 3 4
APPARENT
MW x 10-3
EXT
I P
AP1
LP5
Con
157-
gB-
pgB/g92-
65-
5 6 7 8

FIGURE 55

Comparison of the relative mobility of g92K on gels crosslinked with either N, N'-methylenebisacrylamide (BIS) or N, N'-diallyltartardiamide (DATD). Cells infected with HSV-2 strain HG52 were labelled with [^{35}S]-methionine from 4-26h after infection. An extract was made (EXT) which was used for immunoprecipitation (IP) with two monoclonal antibodies directed against g92K (MABs AP1 and LP5) and a control ascites fluid (Con). Proteins were separated in either a 5 to 12.5% SDS-polyacrylamide gel crosslinked with BIS (lanes 1-4) or a 9% SDS-polyacrylamide gel crosslinked with DATD (lanes 5-8). Some HSV-2-induced proteins have been indicated on the left-hand side of each gel to show the relative mobility of the g92K in each system.

similar to the apparent MW of 124K for the HSV-2 glycoprotein G described by Roizman et al. (1984). Similarities between g92K and gG are discussed in Section 4.8.

DISCUSSION

CHAPTER 4

DISCUSSION

4.1 Sulphation of HSV glycoproteins

One of the objectives of this study was to characterise HSV-induced proteins which are modified by the addition of inorganic sulphate. The results presented show that HSV glycoproteins, like the proteins of many viruses (Erickson and Kaplan, 1973; Pinter and Compans, 1975; Pennington and McCrae, 1977; Pennington et al., 1982) are sulphated. Identification of the major sulphated polypeptide as glycoprotein E was based on two observations. Firstly, it was bound by an Fc-affinity column (fig. 31) and secondly, the range of apparent MWs determined for it (67,000-85,000) is very similar to that (65,000-80,000) determined by Baucke and Spear (1979) for glycoprotein E.

Inorganic sulphate was also incorporated into species which comigrated on one-dimensional gels with gD and gB-1/gC-1. Sulphate label comigrating with gB-2/gC-2 was also occasionally observed (fig. 24). Whether the failure to consistently observe sulphation of gB-2/gC-2 (figs. 24, 29, 31 and 36) reflects reduced levels of synthesis or sulphation of these glycoproteins compared with those of the gB-1/gC-1 is not known.

Since glycoproteins B and C were not adequately resolved from each other on one-dimensional gels, it was not possible to determine whether the sulphate was present on one or both of them. The superior resolution afforded by the technique of two-dimensional PAGE was used to show that gB-1, gC-1 and gD-1 are sulphated (fig. 26). The glycoprotein designated gY by Palfreyman et al. (1983) is also sulphated (fig. 26).

Inorganic sulphate was found to be added at a late stage in the maturation of HSV glycoproteins since it was associated with only the

more mature forms, but not with the precursors of gB, gC and gD. For glycoprotein E, the association was mainly, but not exclusively, with the more fully processed forms (figs. 24, 25, 26, 27, 28, 29, 30, 31 and 33). Since gY is not seen after a 30min. pulse, but appears only after a 2h chase (Palfreyman et al., 1983), it is clear that it requires some maturation steps for its genesis. It therefore follows that like the other HSV-induced glycoproteins, it is sulphated at a late stage.

4.2 Inhibition of the synthesis of host sulphated macromolecules

Synthesis of host sulphated macromolecules is inhibited by wild-type HSV-1. To investigate the mechanism by which inhibition was mediated, use was made of the HSV-1 temperature sensitive mutant, tsK. The lesion in the mutant, tsK, lies an immediate-early (IE) polypeptide of apparent MW 175,000 (IE175) (Preston, 1979a; Murchie, 1982). Cells infected with tsK at the NPT fail to produce most early and late polypeptides (Marsden et al., 1976; Watson and Clements, 1978; Preston, 1979a). The observation that synthesis of host sulphated macromolecules was inhibited at the NPT by tsK (fig. 30) suggests inhibition is mediated by an IE or the early protein (Vmw 136 (143)) and/or virion protein and extends the results of Fenwick and Walker (1978) who showed inhibition of host DNA, RNA and protein synthesis by HSV was caused by a constituent of the virion.

The ability of HSV to reduce synthesis of host-sulphated macromolecules is comparable to that of influenza virus (Nakamura and Compans, 1977) and with pseudorabies virus (Erickson and Kaplan, 1973) in that both viruses markedly inhibit incorporation of sulphate into cellular mucopolysaccharides.

One feature of the gels of uninfected cells labelled with ^{35}S-inorganic sulphate merits comment. The intensity of sulphate labelling appears much greater in one-dimensional gels (figs. 24, 25, 27, 33, 34 and

36) than in two-dimensional gels (fig. 26). This sulphated material is mucopolysaccharide (for review, see Mathews, 1975) which has been fractionated by isoelectric focussing in sucrose gradients into three major and two to four minor components (Podrazky et al., 1970). The major components all have isoelectric points less than 3.80, which is lower than that (4.1) of the most acidic protein which would enter the NEPHGE gel (Marsden et al., 1983). It is likely therefore that the mucopolysaccharides are not seen in 2-D gels because their acidic nature prevents them from entering the NEPHGE gel.

4.3 Physical mapping of the genes encoding the sulphated-proteins gE and 32K, 34K and 35K

Using ^{35}S-inorganic sulphate as a label, it was consistently observed that gE induced by HSV-1 (gE-1) migrated slightly faster on SDS-gels than did gE induced by HSV-2 (gE-2) (figs. 24, 25, 27, 29 and 31). Analysis of the serotype of gE induced by each of the intertypic recombinants and correlation with their genome structures allowed gE to be mapped to the short region of the HSV genome between map co-ordinates 0.832 and 0.95mu (figs. 34 and 35). By using additional intertypic recombinants, each of which had crossovers in the short region of the genome, the right-hand limit for that part of gE which codes for the mobility difference between the two serotypes was placed at 0.935mu.

As shown in fig. 56, the map location of gE is compatible with that obtained independently by Para et al. (1982b) using intertypic recombinants and by Lee et al. (1982a) who mapped the structural gene encoding gE by translation of selected mRNA. More recently, Rixon and McGeoch (1985) have mapped the mRNA for gE-1 and the entire short unique region of the HSV-1 genome has been sequenced and the structural gene encoding gE-1 identified (McGeoch et al., 1985). Since analysis of intertypic recombinants maps that part of the protein which results in the

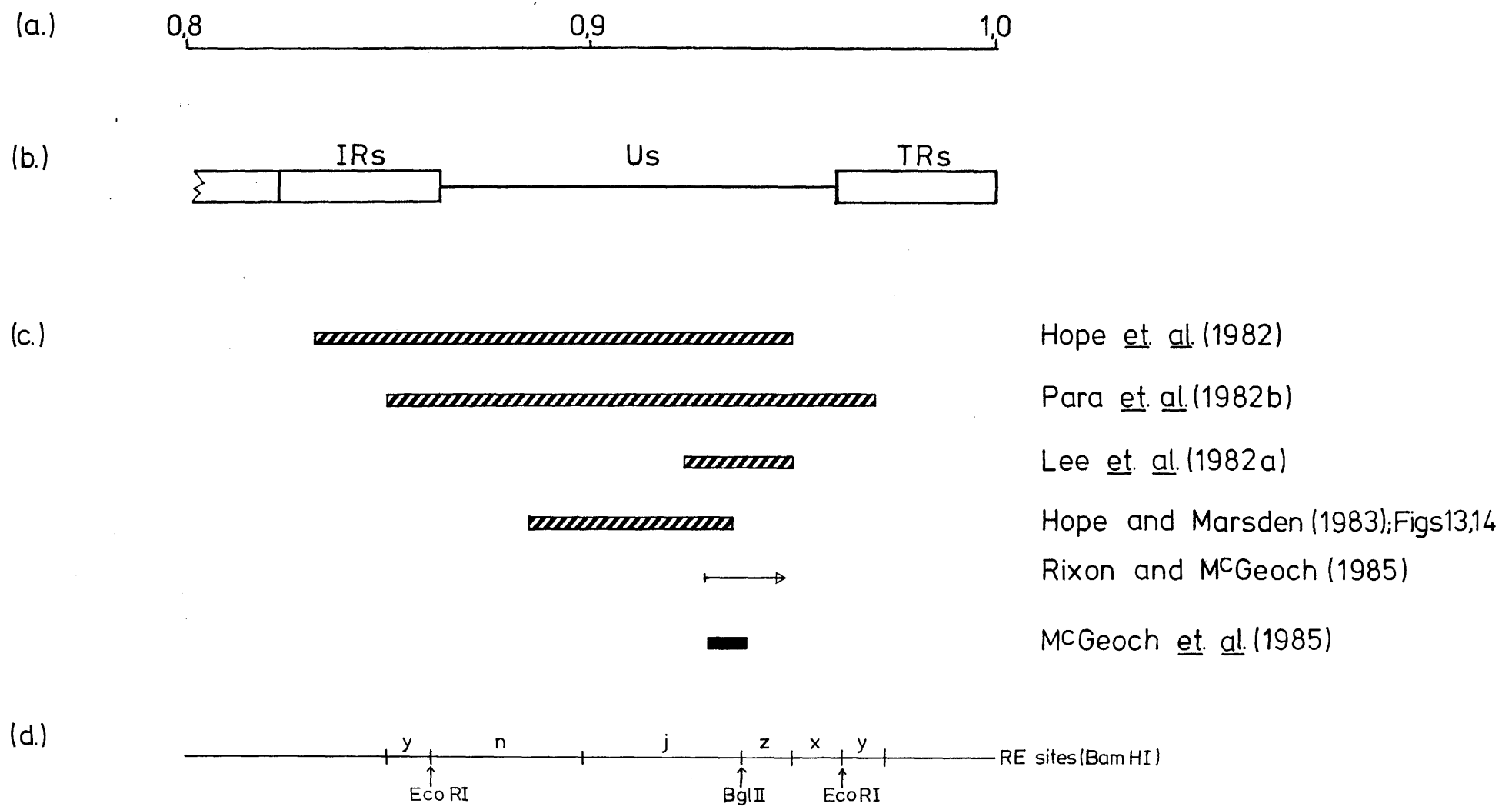

(a.)
0,8
0,9
1,0
(b.)
IRs
Us
TRs
(c.)
Hope et. al. (1982)
Para et. al. (1982b)
Lee et. al. (1982a)
Hope and Marsden (1983); Figs13,14
Rixon and McGeoch (1985)
McGeoch et. al. (1985)
(d.)
y
n
j
z
x
y
RE sites (Bam HI)
Eco RI
Bgl II
Eco RI

FIGURE 56

History of the mapping data for glycoprotein E. The scale 0.8-1.0mu (a) represents only the relevant part of the HSV genome. The short region of the genome is depicted in (b) in which open boxes represent the repeated sequences IR_S and TR_S and the line between the open boxes represents the unique sequences of the genome (U_S). Some relevant restriction enzyme (RE) sites in the HSV-1 genome are shown (d). Published mapping data are shown in (c). Hatched boxes represent the physical mapping limits of gE as determined in those studies. The arrow (⇨) represents the length, position and direction of transcription of mRNA specifying gE and the solid box represents the polypeptide coding sequence.

type-specific mobility difference, examination of fig. 56 allows the conclusion to be made that this region is located in the amino-terminal half of the protein.

The 32K, 34K and 35K proteins, of which over 95% of the total amount synthesised are secreted (Table 12), were mapped according to whether or not they were present in the media of cells infected with intertypic recombinants, since it was observed (figs. 28 and 29) that 17 syn$^+$ -, but not HG52 -, infected cells secreted them. It was clear that every recombinant which induced gE-1, also secreted the 32K, 34K and 35K proteins (figs. 34 and 36). These results demonstrated that the secreted proteins 32K, 34K and 35K are encoded by the same region of the genome, that is, between co-ordinates 0.885 and 0.935mu. However, several other genes which also lie within these co-ordinates, such as the gene encoding gD (Marsden et al., 1978; Ruyechan et al., 1979; Halliburton, 1980; this thesis), gene US4 whose product has been identified as a glycoprotein (M.C. Frame, H.S. Marsden and D.J. McGeoch, submitted for publication), and the putative membrane product of gene US7 (McGeoch et al., 1985) also lie within these co-ordinates and all were therefore possible precursors of the 32K, 34K and 35K proteins. Experiments identifying gE-1 as the precursor were presented in Section 3.8.5 and are discussed in Section 4.6.

4.4 Physical mapping and comparison of nomenclatures used to identify glucosamine-labelled HSV-secreted proteins

Randall et al. (1980) identified six HSV-1 and six HSV-2 polypeptides released from infected cells labelled with glucosamine. The polypeptide profiles on SDS-polyacrylamide gels they presented are very similar to those presented in this thesis (figs. 42 and 54B) and permit a comparison of nomenclatures used to describe their work and this work (Table 9).

Some of the secreted proteins indicated in Table 9 were mapped using intertypic recombinants. The 32K, 34K and 35K proteins are also sulphated and were discussed in Section 4.3. The characterisation of the 92K protein will be presented in Section 4.8.

The HSV-2 40K and the 85K proteins were both mapped to between co-ordinates 0.892 and 0.924mu. It is clear from figs. 29, 38, 39 and 42 that the HSV-2 40K secreted glycoprotein is not sulphated and therefore may not be related to gD or gE, both of which are also encoded within the same region of the genome and are sulphated. Sequence analysis of the U_S region of HSV-2 DNA has shown that HSV-2 encodes a gene US2 (D.J. McGeoch, D. McNab and H.W.M. Rixon, manuscript in preparation) whose predicted translation product has a MW of 32,778 and has a hydrophobic amino terminus. Therefore, gene US2 could possible encode the 40K secreted protein.

The HSV-2 85K and 74K secreted proteins comigrate with the upper and lower forms, respectively, of gE-2 (fig. 27). One of them (85K) was mapped and its position overlapped that of gE. However, unlike gE, both 85K and 74K are secreted (figs. 42 and 54B). These observations suggest that 85K and 74K either are not structurally related to gE-2 or that they are related and part of the population is not sulphated but is secreted.

4.5 Nature of the sulphate and oligosaccharide linkages

The nature of the linkage with which oligosaccharides are attached to glycoproteins was investigated with tunicamycin, a drug known to block the addition of N-linked oligosaccharides (Takatsuki et al., 1975; Tkacz and Lampen, 1975). Experiments with tunicamycin (figs. 32 and 33) confirmed the earlier observations of other investigators (Pizer et al., 1980; Bond et al., 1982; Norrild and Pederson, 1982; Kousoulas et al., 1983) who demonstrated that formation of gB-1, gC-1 and gD-1 was

inhibited by the drug and, showed for the first time, that gE-1 and gY-1 are not produced in the presence of the drug, suggesting that they, like gB-1, gC-1 and gD-1 also contain N-linked oligosaccharides. This suggestion is consistent with the data of Johnson and Spear (1983) who used the enzyme endo-beta-N-acetylglucosaminidase-H, to show that gE-1 contained N-linked oligosaccharides.

Evidence suggesting that inorganic sulphate is attached to N-linked oligosaccharides is provided by the observation that no ^{35}S-labelled inorganic sulphate is incorporated into gB-1, gC-1, gD-1, gE-1 or gY-1 in the presence of tunicamycin (figs. 32 and 33). There is precedence for this type of linkage as Prehm et al. (1979) found that sulphate is esterified to N-linked glycosidic chains of the glycoproteins of the paramyxovirus SV5. Inorganic sulphate may not be attached exclusively to the N-linked oligosaccharides of the HSV-glycoproteins, since incorporation of sulphate into infected cells is not completely inhibited by tunicamycin, but is reduced to only 37% of the untreated control (Table 13). Of particular interest is the accumulation in the presence of the drug of a 70K sulphated polypeptide which could be immunoprecipitated by a monospecific antisera directed against gE-1 (fig. 40) suggesting that the 70K and gE-1 are antigenically related. Since tunicamycin almost completely inhibited the addition of N-linked oligosaccharides (fig. 33, Table 13), it seems probable that the sulphate incorporated into the 70K polypeptide is either attached to O-linked oligosaccharides, as has been found for mucopolysacchaarides (for review, see Sharon and Lis, 1982) or onto the polypeptide backbone, perhaps at the tyrosine position (Huttner, 1982, 1984).

4.6 Origin of the sulphated secreted proteins

It was originally observed (fig. 42; Hope et al., 1982) that a

monoclonal antibody, apparently directed against gD, immunoprecipitated gD, pgD and the 32K, 34K and 35K secreted proteins. This led to the conclusion, at variance with the results presented in this thesis, that the latter were derived from gD. The earlier result can best be explained if the monoclonal antibody producing cell line was a mixture of two clones, one with specificity for gD and the other with specificity for the 32K, 34K and 35K secreted proteins. Unfortunately, this hypothesis cannot be tested as the original cell line is no longer viable. However, hybridoma cells secreting antibodies with an identical mixture of specificities has been independently produced (A. Cross, personal communication).

One piece of evidence which appeared to corroborate the identity of gD and 32K, 34K and 35K was the antigenic data of Randall et al. (1980). As shown in Table 9, these authors demonstrated release of antigens designated a, b, c, d, e and f from HSV-1-infected cells. Two of these, c and e, having apparent MW 50-54K and 30-35K respectively, were precipitated by anti-band II serum (thought to be equivalent to anti-glycoprotein D serum, Norrild, 1980) and are probably the secreted proteins 55K, 57K and 32K, 34K and 35K respectively (Table 9). Two explanations for this observation can be postulated. First, infected cell released polypeptides (ICRPs) c and e are not the secreted proteins 55K, 57K and 32K, 34K and 35K respectively, Second, the anti-band II serum is a mixture of at least two antibody specificities. The second explanation seems more probable.

It was subsequently demonstrated that a monospecific rabbit anti-serum directed against gE-1 could immunoprecipitate the 32K, 34K and 35K secreted proteins (fig. 40) and independently Dr. A. Cross isolated a monoclonal antibody which had specificity for gE but also weakly immunoprecipitated the 32K, 34K and 35K proteins (personal communication).

Corroborative evidence that the 32K, 34K and 35K secreted proteins were derived from gE-1 came from pulse-chase experiments (fig. 41). Glycoprotein E was labelled very intensely with inorganic sulphate during a 2h pulse-label but during a subsequent chase, the label was lost, while there was a concomitant increase in the amount of 32K, 34K and 35K secreted from those infected cells. During this period there was no change in the amount of gD.

The results of tryptic peptide mapping experiments (fig. 44) show conclusively that the secreted proteins 32K, 34K and 35K and also 55K and 57K are encoded by the gE-1 gene.

A surprising aspect arising from examination of the tryptic peptides of gE-1 and the 32K, 34K and 35K is that the peptide maps are identical even though the apparent MWs of the secreted proteins are less than half that of gE-1 (75K). One explanation for the identical patterns would be that at least two of the 32K, 34K, 35K proteins came from different regions of the gE-1 gene and happened to migrate close to each other. However, the individual secreted proteins 32K, 34K and 35K produced identical peptide patterns (fig. 46) demonstrating that they come from the same region of the gE-1 gene.

This puzzling observation would be explained if all the methionine residues in gE-1 were clustered within a fragment wholly contained within the secreted proteins. Examination of the predicted amino acid sequence of gE-1 (McGeoch et al., 1985) shows this explanation is not probable as the methionine residues are distributed along the whole of the structural gene (fig. 57). There are ten methionine residues, the first of which would be removed with the putative signal peptide. Two residues lie within the putative membrane anchoring sequence of gE-1 which would be expected to be absent from the secreted proteins. A plausible mechanism accounting for the identical tryptic peptide fingerprints could be postulated if the

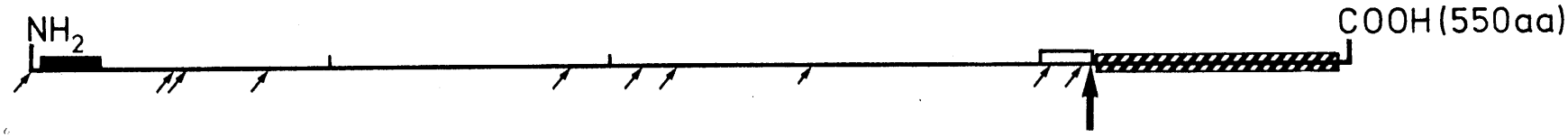
putative membrane anchoring sequence is not functional and that gE-1 is held in the membrane by some other mechanism perhaps via its covalent linkage to fatty acid (Johnson and Spear, 1983). If such linkage was at the position indicated in fig. 57 and if cleavage of that region (large arrow fig. 57) allowed secretion of the truncated protein, then all the methionine residues would be included in a secreted, truncated form of gE-1. How then to account for the large difference in mobility of gE-1 and the secreted proteins 32K, 34K, 35K? Possibly the fatty acids attached to the carboxy region severely retard migration in the gel. Another explanation for the observed identical tryptic peptide fingerprints is that some peptides of gE-1 are unstable and are degraded during preparation.

The behaviour of gE-1 during a pulse and subsequent chase is compatible with both explanations. Following a 2h pulse during which gE-1 is labelled, the amount of gE-1 decreases during the chase and the secreted proteins are generated. However, the remainder of gE-1 cannot be detected, suggesting that this portion of gE-1 either contains no radioactive label or is degraded.

4.6.1 Evidence for involvement of a serum component in the generation of the secreted proteins

The 55K, 57K, 32K, 34K, and 35K proteins could be generated (i) from an extract of HSV-1-infected cells by passing the extract through an Fc-affinity column (fig. 47); (ii) in vitro, by mixing purified gE-1 with serum (fig. 48) and (iii) in tissue culture, but only in the presence of serum (fig. 49). These data demonstrate that an unidentified component in calf, non-immune rabbit and human serum is responsible for their generation and that their production occurs after translation. The component is not IgG since addition of purified IgG did not generate the 32K, 34K, 35K proteins (data not shown).

Interestingly, the addition of human serum to the infected cell


NH2
COOH(550aa)

monolayers generated principally the 55K and 57K proteins and much lower amounts of the 32K, 34K and 35K proteins than was found with rabbit or calf sera (figs. 48 and 49). If the 55K and 57K and the 32K, 34K and 35K proteins were generated by a single enzyme common to all three sera then the two groups of proteins would be expected to be generated in the same ratio by all sera. Since that expectation was not met experimentally, other mechanisms must be postulated. It is possible that one enzyme is responsible for producing the 55K and 57K and a second enzyme is responsible for producing the 32K, 34K and 35K proteins and that human serum is relatively deficient in the second enzyme. A second possibility is that a single enzyme is responsible for generating both groups of proteins but that the proteins of each of the three species have slightly different preferred modification sites.

The mechanism by which the secreted proteins are generated from gE-1 is unknown. One likely possibility would be cleavage but this remains to be investigated.

The role of serum proteins in the maturation of viral glycoproteins has been previously demonstrated. Proteolytic cleavage of the haemagglutinin glycoprotein of influenza virus is necessary for enhancement of infectivity (Lazarowitz and Choppin, 1975). This cleavage involves the enzyme plasmin, which is derived from the serum protein plasminogen (Lazarowitz et al., 1973).

4.7 Possible biological functions of the sulphated secreted proteins and the serum components responsible for their production

Whatever role, if any, the 32K, 34K and 35K and the 55K and 57K sulphated proteins have during HSV-1 infection, it seems pertinent to initially speculate about their function in relation to gE-1, the protein from which they are derived.

Fc-binding activity has been demonstrated on cells infected with three different herpesviruses, HSV (Yasuda and Milgrom, 1968) of which gE has been shown to be the Fc-receptor (Baucke and Spear, 1979; Para et al., 1980, 1982a), CMV (Keller et al., 1976; Rahman et al., 1976; Westmoreland et al., 1976) and VZV (Ogata and Shigeta, 1979). Moreover there is homology between the VZV gene 68 and gE of HSV (Davison and Scott, manuscript in preparation). However, it has been demonstrated (Ishak et al., 1984) that several low-passage isolates of VZV did not induce the appearance of Fc-receptors. Ishak et al. (1984) speculated that the genetic message for Fc-receptor induction is present in the VZV genome but, in low-passaged isolates, it is rendered inactive or is expressed at a very low frequency compared to Fc-receptor induction in high-passaged isolates as used by Ogata and Shigeta (1979). However, they did not test their speculation. The physiological role of the herpesvirus induced Fc-receptors has not been elucidated.

Costa et al. (1977) showed that the presence of high concentrations of Fc-fragments from rabbit IgG can suppress HSV production in tissue and it has been demonstrated in vitro (Adler et al., 1978) that Fc-receptors can play a role in protecting HSV-1-infected cells against immune cytolysis, but whether they play a role in vivo during infection remains to be tested.

Another function in which Fc-receptors have been implicated is the maintenance of latency. Stevens and Cook (1974) showed that latently-infected ganglia transplanted to non-immune mice allowed the replication of HSV whereas immunized mice had significantly less virus as determined by antigen expression and virus DNA synthesis in the transplanted ganglia. To explain this result, Lehner et al. (1975) suggested on an entirely hypothetical basis that the antiviral IgG binds simultaneously to the viral antigens and Fc-receptors expressed on the infected cell surface and

therefore the cells in which HSV establishes a latent infection may not be accessible to the effector cells involved in immune cytotoxicity. Recently Johansson et al. (1985) demonstrated that the HSV-1 Fc-receptor does not bind the Fc region of mouse IgG. If the Fc-receptor is important in host-cell interactions in any way, this difference (the affinity for the HSV Fc-receptor between immunoglobulins from different animal orders) could give rise to major misinterpretations of such experiments.

None of the sulphated secreted proteins retain Fc-binding affinity. Furthermore, it is not known whether Fc-binding affinity is destroyed perhaps due to conformational changes in the polypeptide or cleavage within the amino acid-sequence which comprises the Fc-receptor or whether Fc-binding affinity remains in another polypeptide fragment other than those detected.

Fc-receptors may affect the fate of infected cells. Antibody-dependent, complement mediated lysis of infected cells is initiated via the CIq protein of complement component 1(CI) binding to bound-antigen-specific antibody. It is possible that gE-1 depletes the number of IgG molecules available to bind to their target antigens, thus favouring survival of the virus.

The sulphated polypeptides are only a subset of the total polypeptides secreted from HSV-1-infected cells (figs. 42 and 53B). Since many of them are glycosylated and glycoproteins are among the major antigenic determinants on the infected cell membrane, it is possible that polypeptides which are preferentially secreted from HSV-infected cells "mop-up" HSV-specific antibodies, thus favouring survival of the virion and the infected cells.

4.7.1 Future experiments

The data presented in this study have answered some of the

questions posed at the start and have in turn generated others. Experiments are suggested below to approach some of these new questions. Purification of gE-1 and the secreted proteins 32K, 34K and 35K and 55K and 57K, together with amino acid sequence studies would identify the region of gE-1 from which the secreted proteins are derived. Although a serum protein is implicated in the genesis of the 32K, 34K, 35K, 55K and 57K proteins, the mechanism responsible remains obscure. It is most likely one of cleavage but experiments to test this possibility have yet to be performed. These experiments might involve infecting cells in the presence or absence of protease inhibitors such as TPCK or TLCK or monoclonal antibodies directed against the serum proteases. A better understanding of the putative cleavage mechanism of gE-1 would aid in studies examining whether gE-1 is involved in immune cytolysis and whether its Fc-receptor and the secreted proteins are involved in these immune mechanisms. Mutants in gE generated by in vitro mutagenesis may help to understand the function of this glycoprotein during infection.

4.8 Characterisation of the HSV-2 g92K

The glycoprotein designated g92K was originally identified by Marsden et al. (1978) who mapped the gene encoding it to within the short region of the HSV-2 genome (0.830 to 0.950mu). Data presented in this thesis and Marsden et al. (1984) refine this location to between co-ordinates 0.892 and 0.924mu.

Four lines of evidence suggested that g92K was a new glycoprotein, distinct from gB, gC, gD and gE. First, the genomic location of g92K is not compatible with that of gB or gC, both of which map in the long unique region of the genome (see fig. 58) (Frink et al., 1983; Halliburton, 1980; Marsden et al., 1978; Para et al., 1983; Ruyechan et al., 1979). Second, the carbohydrate composition and extent of sulphation of g92K

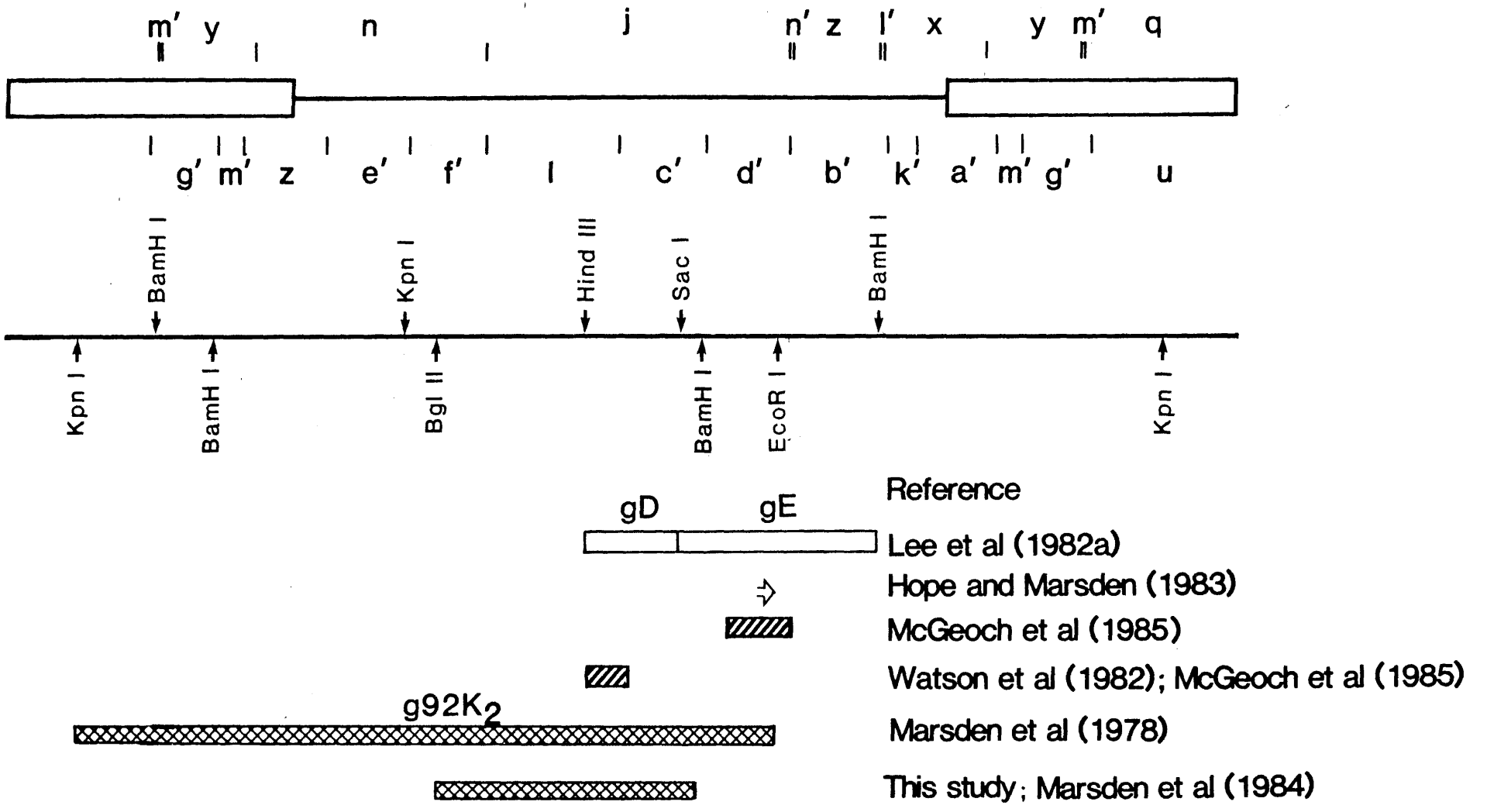

m′ y n j n′ z l′ x y m′ q
g′ m′ z e′ f′ l c′ d′ b′ k′ a′ m′ g′ u
BamH I
Kpn I
Hind III
Sac I
BamH I
Kpn I
BamH I
Bgl II
BamH I
EcoR I
Kpn I
gD
gE
g92K2
Reference
Lee et al (1982a)
Hope and Marsden (1983)
McGeoch et al (1985)
Watson et al (1982); McGeoch et al (1985)
Marsden et al (1978)
This study; Marsden et al (1984)

FIGURE 58

Map location of the HSV-2-induced g92K. The figure illustrates the short region of the genome. The upper section shows the BamHI sites in both HSV-1 DNA (upper letter) and HSV-2 DNA (lower letters). Below this are the restriction enzyme sites which delimit the various glycoproteins: HSV-2 KpnI, a-r; HSV-1 BamHI, q-m', HSV-2 BamHI, g'-m'; HSV-1 KpnI, j-h; HSV-2 BglII, q-l; HSV-1 HindIII, g-n; the middle SacI site in BamHI, j; HSV-2 EcoRI, n-o; HSV-1 BamHI, z-x; HSV-2 KpnI, a-r. The genomic location of gD-1 and gE-1 obtained by mRNA selection and in vitro translation (Lee et al., 1982a) are shown. The open arrow denotes the right-hand limit of that part of the glycoprotein E gene coding for the difference in mobility between the two serotypes (Hope and Marsden, 1983), the hatched region shows the polypeptide coding sequence for gE-1 (McGeoch et al., 1985; Rixon and McGeoch, 1985) and gD-1 (Watson et al., 1982; McGeoch et al., 1985), respectively. The cross-hatched region shows the location obtained for g92K (Marsden et al., 1978, 1984; this study).

differed from that of glycoproteins D or E (fig. 27). Third, the recombinant R12-1 did not induce g92K, but did induce gE-2 (fig. 53) showing that g92K is encoded, at least in part, by a different region of the genome from that encoding gE. Fourth, two clonally unrelated monoclonal antibodies reacted with g92K and not with any of the known processed forms of gB, gC, gD or gE. Thus, g92K is distinct from all other known glycoproteins by at least two of the above lines of evidence.

Recently Roizman et al. (1984) have re-evaluated their mapping of gC-2 (Ruyechan et al., 1979). They now consider their earlier mapping to be incorrect and now place it in U_S overlapping with the position of g92K. They redefined the glycoprotein as that reactive with monoclonal antibody H966 and re-designated it gG.

It is possible that gG and g92K are one and the same, since besides having overlapping map positions, they are similar in that no equivalent HSV-1 glycoprotein had at that time been identified. Furthermore, the MWs of both glycoproteins are compatible. gG has an apparent MW of 124K on gels crosslinked with DATD which is comparable with that observed in this study (120K, fig. 55) for g92K on gels crosslinked with DATD.

Recently it was observed (Olofsson et al., submitted for publication) using monoclonal antibody, LP5, specific for g92K and H966 specific for gG, that these two glycoproteins share the property of being the only HSV-2 glycoproteins with affinity for helix pomatia lectin. It therefore seems beyond reasonable doubt that g92K and gG-2 are one and the same glycoprotein.

Other investigators who have described g92K but, who failed to recognise it as a novel glycoprotein, include Balachandran et al. (1982b), who isolated a monoclonal antibody 13 alpha C5 which immunoprecipitated a glycoprotein of apparent MW 130K. More recently Balachandran and

Hutt-Fletcher (1985) showed that the glycoproteins immunoprecipitated with monoclonals 13 alpha C5 and AP1 (which also defines g92K (Marsden et al., 1984)) have identical MWs and concluded that they are one and the same and designated it gG-2. However, it seems pertinent to emphasise that these authors did not use the monoclonal which defined gG-2 (i.e. monoclonal H966). Therefore, their studies only demonstrate identity between g92K and their glycoprotein. The studies of Olofsson et al. (in press) are necessary for establishment of identity between their glycoprotein and gG-2.

Evidence is accumulating concerning the synthesis and processing of g92K. That it continues to accumulate late into infection (fig. 50) was confirmed by Balachandran et al. (1982b) using the monoclonal antibody 13 alpha C5.

Balachandran and Hutt-Fletcher (1985) presented evidence to suggest that g92K undergoes proteolytic cleavage of a partially glycosylated precursor during its maturation and also showed that in the presence of tunicamycin, g92K did not accumulate, but that polypeptides which were resistant to endo-H (which cleaves high-mannose oligosaccharides) and endoglycosidase-F (which cleaves both high-mannose and complex oligosaccharides from asparagine) did. One of these polypeptides labelled with [^{3}H]-galactose and [^{3}H]-glucosamine in the presence of tunicamycin, suggesting that g92K contains O-linked oligosaccharides. This suggestion was corroborated by the evidence of Olofsson et al. (in press) who showed that g92K/gG-2 is the only HSV-2 induced glycoprotein that detectably binds to helix pomatia lectin, demonstrating that it contains O-linked oligosaccharides (since helix pomatia lectin has affinity for N-acetyl galactosamine - a sugar found only in O-linked oligosaccharides) and by Serafini-Cessi et al. (1985) who demonstrated the presence of three size classes of O-linked

oligosaccharides on gG-2.

As discussed earlier (Section 4.4), several investigators have reported glycoproteins to be secreted from HSV-infected cells (Chen et al., 1978; Kaplan et al., 1975; Norrild and Vestergaard, 1979; Randall et al., 1980; Hope et al., 1982). Here it is demonstrated that g92K secreted from infected cells corresponds to the intracellular g92K (fig. 54). A protein of this apparent MW was shown by Randall et al. (1980) to be secreted from HSV-2-infected cells and was designated ICRP-1. As discussed in Section 4.4, ICRP-1 is probably g92K. Also, Norrild et al. (1979) described an HSV-2 specific glycoprotein, designated Ag4A which was the major glucosamine-labelled antigen secreted from infected cells and was not antigenically related to any of the other proteins. It seems likely that g92K corresponds to Ag4A.

Recently, when preparation of this thesis was almost complete, DNA sequencing studies showed that the HSV-1 gene, US4 (McGeoch et al., 1985) shares homology with an HSV-2 putative gene sequenced by D.J. McGeoch, D. McNab and H.W.M. Rixon (manuscript in preparation). Antibodies generated in rabbits against a synthetic oligopeptide corresponding to a stretch of amino acids from an internal hydrophilic region of the sequence of the predicted HSV-1 US4 gene product precipitates three glycoprotein species of apparent MW 39,000, 48,000 and 56,000 (Frame et al., submitted for publication). The relationship between these three glycoproteins and the structural gene of HSV-1 US4 remains to be elucidated. An oligopeptide corresponding to a region of amino acids conserved between the HSV-1 US4 gene product and HSV-2 gG precipitated both gene products (M. Frame, personal communication). It can be concluded that the product of US4 should be known as gG-1.

The HSV-1 g88K identified in figs. 51, 53 and 54 was thought to be a potential candidate for the HSV-1 equivalent of g92K (Marsden et al.,

1984) since it mapped to the corresponding region on the HSV-1 genome and no recombinants induced both g88K and g92K. Clearly none of the three glycoprotein species synthesised from HSV-1 gene US4 correspond to g88K. However, analysis of the nucleotide sequence of the HSV-1 U_S region in which g88K maps shows that gene US7, whose predicted product is 41,366, is a probable candidate to encode g88K. This possibility remains to be investigated.

REFERENCES

ADDISON,C.,RIXON,F.J.,PALFREYMAN.J.W.,O,HARA,M.and PRESTON,V.G(1984).Characterisation of a herpes simplex virus type 1 mutant which has a temperature sensitive defect in penetration of cells and assembly of capsids. Virology,138,246-259.

REFERENCES

ACHORD, D.T., BROT, F.E., BELL, C.E. and SLY, W.S. (1978). Human beta-glucuronidase - *in vivo* clearance and *in vitro* uptake by a glycoprotein recognition system on reticulo endolethial cells. Cell 15, 269-278.

ADLER, R., GLORIOSO, J.C., COSSMAN, J. and LEVINE, M. (1978). Possible role of Fc receptors on cells infected and transformed by herpesvirus: Escape from immune cytolysis. Infect. Immun. 21, 442-447.

AL-SAADI, S.A., CLEMENTS, G.B. and SUBAK-SHARPE, J.H. (1983). Viral genes modify herpes simplex virus latency both in mouse footpad and sensory ganglia. J. Gen. Virol. 64, 1,175-1,179.

ALWINE, J.C., STEINHART, W.L. and HILL, C.W. (1974). Transcription of herpes simplex virus type 1 DNA in nuclei from infected HEp-2 and KB cells. Virology 60, 302-307.

ANDERSON, B., SENO, N., SAMPSON, P., RILEY, J.G., HOFFMAN, P. and MEYER, K. (1964). Threonine and serine linkages in mucopolysaccharides and glycoproteins. J. Biol. Chem. 239, 2716-2717.

ANDERSON, C.W., BAUM, P.R. and GESTELAND, R.F. (1973). Processing of adenovirus 2-induced proteins. J. Virol. 12, 241-252.

ANDERSON, D.J., WALTER, P. and BLOBEL, G. (1982). Signal recognition protein is required for the integration of acetylcholine receptor δ subunit, a transmembrane glycoprotein, into the endoplasmic reticulum. J. Cell Biol. 93, 501-506.

ANDERSON, K.P., COSTA, R.H., HOLLAND, L.E. and WAGNER, E.K. (1980). Characterization of herpes simplex virus type 1 RNA present in the absence of de novo protein synthesis. J. Virol. 34, 9-27.

ANDERSON, K.P., FRINK, R.J., DEVI, G.B., GAYLORD, B.H., COSTA, R.H. and WAGNER, E.K. (1981). Detailed characterization of the mRNA mapping in the *Hind*III fragment K region of the herpes simplex virus type

1 genome. J. Virol. 37, 1,011-1,027.

ANDERSON, K.P., STRINGER, J.R., HOLLAND, L.E. and WAGNER, E.K. (1979). Isolation and localization of herpes simplex virus type 1 mRNA. J. Virol. 30, 805-820.

ASHER, Y., HELLER, M. and BECKER, Y. (1969). Incorporation of lipids into herpes simplex virus particles. J. Gen. Virol. 4, 65-76.

ATKINSON, M.A., BARR, S. and TIMBURY, M.C. (1978). The fine structure of cells infected with temperature-sensitive mutants of herpes simplex type 2. J. Gen. Virol. 40, 103-119.

AXELSSON, B., KIMURA, A., HAMMERSTROM, S., WIGZELL, H., NILSSON, K. and MELLSTEDT, H. (1978). Helix pomatia A haemagglutinin: selectivity of binding to lymphocyte surface glycoproteins on T cells and certain B cells. Eur. J. Immunol. 8, 757-764.

BACCHETTI, S., EVELEGH, M.J., MUIRHEAD, B., SARTORI, C.S. and HUSZAR, D. (1984). Immunological characterization of herpes simplex virus types 1 and 2 polypeptide(s) involved in viral ribonucleotide reductase activity. J. Virol. 49, 591-593.

BACHENHEIMER, S.L. and ROIZMAN, B. (1972). Ribonucleic acid synthesis in cells infected with herpes simplex virus. VI. Polyadenylic acid sequences in viral messenger ribonucleic acid. J. Virol. 10, 875-879.

BAENZIGER, J. and KORNFELD, S. (1974). Structure of the carbohydrate units of IgE immunoglobulin. II. Sequence of the sialic acid-containing glycopeptides. J. Biol. Chem. 249, 1,897-1,903.

BAHL, O.P. (1970). Glycosidases of Aspergillus niger. II. Purification and general properties of 1-2-alpha-L-fucosidase. J. Biol. Chem. 245, 299-304.

BALACHANDRAN, N., BACCHETTI, S. and RAWLS, W.E. (1982a). Protection against lethal challenge of BALB/C mice by passive transfer of monoclonal antibodies to five glycoproteins of herpes simplex virus type 2. Infect. Immun. 37, 1,132-1,137.

BALACHANDRAN, N., HARNISH, D., KILLINGTON, A., BACCHETTI, S. and RAWLS, W.E. (1981). Monoclonal antibodies to two glycoproteins of herpes simplex virus type 2. J. Virol. 39, 438-446.

BALACHANDRAN, N., HARNISH, D., RAWLS, W.E. and BACCHETTI, S. (1982b). Glycoproteins of herpes simplex virus type 2 as defined by monoclonal antibodies. J. Virol. 44, 344-355.

BALACHANDRAN, N. and HUTT-FLETCHER, L.M. (1985). Synthesis and processing of glycoprotein G of herpes simplex virus type 2. J. Virol. 54, 825-832.

BANKS, L., PURIFOY, D.J.M., HURST, P.-F., KILLINGTON, R.A. and POWELL, K.L. (1983). Herpes simplex virus non-structural proteins. IV. Purification of the virus-induced deoxyribonuclease and characterization of the enzyme using monoclonal antibodies. J. Gen. Virol. 64, 2,249-2,260.

BARTOSKI, M.J. and ROIZMAN, B. (1976). RNA synthesis in cells infected with herpes simplex virus. XII. Differences in the methylation patterns of viral RNA during the reproductive cycle. J. Virol. 20, 583-588.

BATTERSON, W. and ROIZMAN, B. (1983). Characterization of the herpes simplex virion-associated factor responsible for the induction of alpha genes. J. Virol. 46, 371-377.

BAUCKE, R.B. and SPEAR, P.G. (1979). Membrane proteins specified by herpes simplex viruses. V. Identification of an Fc-binding glycoprotein. J. Virol. 32, 779-789.

BAYLISS, G.J., MARSDEN, H.S. and HAY, J. (1975). Herpes simplex virus proteins: DNA-binding proteins in infected cells and in the virus structure. Virology 68, 124-134.

BECKER, Y., CHEJANOVSKY, N. and FRIDLANDER, B. (1980). DNA-binding proteins in the nuclei of herpes simplex virus-infected, arganine-deprived, BSC-1 cells. J. Gen. Virol. 50, 259-267.

BECKER, Y., DYM, H. and SAROV, I. (1968). Herpes simplex virus DNA. Virology 36, 184-192.

BEDI, G.S., FRENCH, W.C. and BAHL, O.P. (1982). Structure of carbohydrate units of ovine luteinizing hormone. J. Biol. Chem. 257, 4,345-4,355.

BENOIST, C., O'HARE, K., BREATHNACH, R. and CHAMBON, P. (1980). The ovalbumin gene-sequence of putative control regions. Nucl. Acids Res. 8, 127-142.

BEN-PORAT, T. and KAPLAN, A.S. (1962). The chemical composition of herpes simplex and pseudorabies viruses. Virology 16, 261-266.

BEN-PORAT, T. and RIXON, F.J. (1977). Replication of herpesvirus DNA. IV. Analysis of concatemers. Virology 94, 61-70.

BEN-PORAT, T. and TOKUZEWSKI, S.A. (1977). Replication of herpesvirus DNA. II. Sedimentation characteristics of newly synthesised DNA. Virology 79, 292-301.

BEN-PORAT, T. and VEACH, R.A. (1980). Origin of replication of the DNA of a herpesvirus (pseudorabies). PNAS 77, 172-175.

BEN-ZEEV, A., ASHER, Y. and BECKER, Y. (1976). Synthesis of herpes simplex virus-specified RNA by an RNA polymerase II in isolated nuclei in vitro. Virology 71, 302-311.

BERGMAN, J.E. and SINGER, S.J. (1983). Immunoelectron microscopic studies of the intracellular transport of the membrane glycoprotein (G) of vesicular stomatitis virus in infected Chinese hamster ovary cells. J. Cell Biol. 97, 1,777-1,787.

BERGMAN, L.W. and KUEHL, W.M. (1978). Temporal relationship of translation and glycosylation of immunoglobulin heavy and light chains. Biochemistry 17, 5,174-5,180.

BEYER, T.A. and HILL, R.L. (1982). Glycosylation pathways in the biosynthesis of non-reducing terminal sequences in oligosaccharides of glycoproteins. In: "The Glycoconjugates". Ed. M.I. Horowitz, Volume III, pp.25-45.

BEYER, T.A., SADLER, J.E., REARICK, J.I., PAULSON, J.C. and HILL,

R.L. (1981). Glycosyltransferases and their use in assessing oligosaccharide structure and structure-function relationships. Adv. in Enzymology, 52, 23-175.

BISWAL, N., FELDAN, P. and LEVY, C.C. (1983). A DNA topoisomerase activity co-purifies with the DNA polymerase induced by herpes simplex virus. Biochimica et biophysica acta. 740, 379-389.

BLOBEL, G. (1980). Intracellular protein topogenesis. PNAS 77, 1,496-1,500.

BLOBEL, G. and DOBBERSTEIN, B. (1975). Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. J. Cell Biol. 67, 835-851.

BLOMBERG, J. (1979). Studies on the attachment of herpes simplex virus. Effect of trypsin treatment. Arch. Virol. 61, 201-206

BLUE, W.T. and STOBBS, D.G. (1981). Isolation of a protein kinase induced by herpes simplex virus type 1. J. Virol. 38, 383-388.

BOEKE, J.D. and MODEL, P. (1982). A prokaryotic membrane anchor sequence: carboxyl terminus of bacteriophage f1 gene III protein retains it in the membrane. PNAS 79, 5,200-5,204.

BOHAK, Z. (1969). Purification and characterisation of chicken pepsinogen and chicken pepsin. J. Biol. Chem. 244, 4,638-4,648.

BOND, V.C. and PERSON, S. (1984). Fine structure physical map locations of alterations that affect cell fusion in herpes simplex virus type 1. Virology 132, 368-376.

BOND, V.C., PERSON, S. and WARNER, S.C. (1982). The isolation and characterization of mutants of herpes simplex virus type 1 that induce cell fusion. J. Gen. Virol. 61, 245-254.

BOOKOUT, J.B. and LEVY, C.C. (1980). Comparative examination of the polypeptides of herpes simplex virus: types 1 and 2. Virology 101, 198-216.

BOSMANN, H.B., HAGOPIAN, A. and EYLAR, E.H. (1968). Glycoprotein biosynthesis: the characterization of two glycoprotein: fucosyltransferases in HeLa cells. Arch. Biochem. Biophys. 128, 470-481.

BOURKES, A.E. and MENEZES, J. (1979). Studies on the induction of Fc-receptors and synthesis of IgM in primary and chronically infected lymphoid (Raji) cells by herpes simplex virus. J. Gen. Virol. 44, 361-371.

BREATHNACH, R. and CHAMBON, P. (1981). Organisation and expression of eukaryotic split genes coding for proteins. Ann. Rev. Biochem. 50, 349-383.

BROWN, S.M., RITCHIE, D.A. and SUBAK-SHARPE, J.H. (1973). Genetic studies with herpes simplex virus type 1. The isolation of temperature sensitive mutants, their arrangement into complementation groups and recombination analysis leading to a linkage map. J. Gen. Virol. 18, 329-346.

BUCKMASTER, E.A., GOMPELS, U. and MINSON, A. (1984). Characterisation and physical mapping of an HSV-1 glycoprotein of approximately 115×10^3 molecular weight. Virology 139, 408-413.

BURK, R.R. (1967). In: "Growth regulating substances from animal cells in culture". p.39. Eds. V. Defendi and M.P.G. Stoker, Philadelphia, Pa, Wistar Institute Press.

BURKE, D.J. and KEEGSTRA, K. (1976). Purification and composition of the proteins from sindbis virus grown in chick and BHK cells. J. Virol. 20, 676-686.

BUSBY, D.W.G., HOUSE, W. and MACDONALD, J.R. (1964). In: "Virological Techniques" published by Churchill, London.

BUSS, J.E. and SEFTON, B.M. (1985). Myristic acid, a rare fatty acid, is the lipid attached to the transforming protein of Rous Sarcoma Virus and its cellular homolog. J. Virol. 53, 7-12.

BUTTERS, T.D. and HUGHES, R.C. (1980). Effect of tunicamycin on insect cells. Biochem. Soc. Trans. 8, 170-171.

BZIK, D.J., FOX, B.A., DELUCA, N.A. and PERSON, S. (1984a). Nucleotide sequence specifying the glycoprotein gene, gB, of herpes simplex virus type 1. Virolfogy 133, 301-314.

BZIK, D.J., FOX, B.A., DELUCA, N.A. and PERSON, S. (1984b). Nucleotide sequence of a region of the herpes simplex virus type 1 gB glycoprotein gene: mutations affecting rate of entry and cell fusion. Virology 137, 185-190.

CABRAL, G.A. and SCHAFFER, P.A. (1976). Electron microscope studies of temperature sensitive mutants of herpes simplex virus type 2. J. Virol. 18, 727-737.

CAMPADELLI-FIUME, G., POLETTI, L., DALL, OLIO, F. and SERAFINI-CESSI, F. (1982). Infectivity and glycoprotein processing of herpes simplex virus type 1 grown in a ricin-resistant cell line deficient in N-acetylglucosaminyl transferase 1. J. Virol. 43, 1,061-1,071.

CAMPBELL, M.E.M., PALFREYMAN, J.W. and PRESTON, C.M. (1984). Identification of herpes simplex virus DNA sequences which encode a transacting polypeptide responsible for stimulation of immediate early transcription. J. Mol. Biol. 180, 1-19.

CAMPBELL, W.F., MURRAY, B.K., BISWAL, N. and BENYESH-MELNICK, M. (1974). Restriction of herpes simplex virus type 1 replication in oncornavirus-transformed cells. J. Nat. Cancer Inst. 74, 757-761.

CARADONNA, S.J. and CHENG, Y.C. (1981). Induction of uracil-DNA glycosylase and dUTP nucleotidohydrolase activity in herpes simplex virus-infected human cells. J. Biol. Chem. 256, 9,834-9,837.

CARLSON, D.M., JOURDIAN, G.W. and ROSEMAN, S. (1973). The sialic acids. XIV. Synthesis of sialyl-lactose by sialyltransferase from rat mammary gland. J. Biol. Chem. 248, 5,742-5,750.

CARSTEN, M.E. and PIERCE, J.G. (1963). Chemical studies on thyrotropin preparations and related pituitary glycoproteins. J. Biol. Chem. 238, 1,724-1,731.

CARTER, V.C., JENNINGS, S.R., RICE, P.L. and TREVETHIA, S.S. (1984). Mapping of a herpes simplex virus type 2 encoded function that affects the susceptibility of herpes simplex virus-infected target cells to lysis by herpes simplex virus-specific cytotoxic T lymphocytes. J. Virol. 49, 766-771.

CARTER, V.C., RICE, P.L. and TREVETHIA, S.S. (1982). Intratypic and intertypic specificity of lymphocytes involved in the recognition of herpes simplex virus glycoproteins. Infect. Immun. 37, 116-126.

CASH, P., HENDERSHOT, L. and BISHOP, D.H.L. (1980). The effects of glycosylation inhibitors on the maturation and intracellulalr polypeptide synthesis induced by Snowshoe Hare Bunyavirus. Virology 103, 235-240.

CASSAI, E., MANSERVIGI, R., CORALLINI, A. and TERNI, M. (1976). Plaque dissociation of herpes simplex viruses: biochemical and biological characters of the viral variants. Intervirology 6, 212-223.

CASSAI, E.N., SARMIENTO, M. and SPEAR, P.G. (1975). Comparison of the virion proteins specified by herpes simplex virus types 1 and 2. J. Virol. 16, 1,327-1,331.

CASSIDY, J.T., JOURDIAN, G.W. and ROSEMAN, S. (1965). The sialic acids. VI. Purification and properties of sialidase from Clostridium perfringens. J. Biol. Chem. 240, 3,501-3,506.

CHAI, L. (1971). Architecture of the DNA molecule within Epstein-Barr virus (EBV). PNAS 74, 1,734-1,738.

CHAMPOUX, J.J. (1978). Proteins that affect DNA conformation. Ann. Rev. Biochem. 47, 449-479.

CHAN, W. (1983). Protective immunization of mice with specific HSV-1 glycoproteins. Immunology 49, 343-352.

CHANG, C.N., MODEL, P. and BLOBEL, G. (1979). Membrane biogenesis: cotranslational integration of the bacteriophage f1 coat protein into an Escherichia coli membrane fraction. PNAS 76, 1,251-1,255.

CHAPMAN, A. and KORNFELD, R. (1979a). Structure of the high

mannose oligosaccharide of a human IgM myeloma protein. I. The major oligosaccharide of the two high mannose glycopeptides. J. Biol. Chem 254, 816-823.

CHAPMAN, A. and KORNFELD, R. (1979b). Structure of the high mannose oligosaccharide of a human IgM myeloma protein. II. The minor oligosaccharides of high mannose glycopeptide 1. J. Biol. Chem. 254, 824-828.

CHARTRAND, P., CRUMPACKER, C.S., SCHAFFER, P.A. and WILKIE, N.M. (1980). Physical and genetic analysis of the herpes simplex virus DNA polymerase locus. Virology 103, 311-326.

CHARTRAND, P., STOW, N.D., TIMBURY, M.C. and WILKIE, N.M. (1979). Physical mapping of paa^r mutants of herpes simplex virus type 1 and type 2 by intertypic marker rescue. J. Virol. 31, 265-276.

CHARTRAND. P., WILKIE, N.M. and TIMBURY, M.C. (1981). Physical mapping of temperature-sensitive mutations of herpes simplex virus type 2 by marker rescue. J. Gen. Virol. 52, 121-133.

CHEN, A.B., BEN-PORAT, T., WHITLEY, R.J. and KAPLAN, A.S. (1978). Purification and characterization of proteins excreted by cells infected with herpes simplex virus and their use in diagnosis. Virology 91, 234-242.

CHENG, Y.-C. and OSTRANDER, M. (1976). Deoxythymidine kinase induced in HeLa TK$^-$ cells by herpes simplex virus types I and II. J. Biol. Chem. 251, 2,605-2,610.

CHOU, P.Y. and FASMAN, G.D. (1974). Prediction of protein conformation. Biochemistry 13, 222-245.

CHURCHILL, A.E. and BIGGS, P.M. (1967). Agent of marek's disease in tissue culture. Nature 215, 528-530.

CINES, D.B., LYSS, A.P., BINA, M., CORKEY, R., KEFALIDES, N.A. and FRIEDMAN, H.M. (1982). Fc and C3 receptors induced by herpes simplex virus on cultured human endothelial cells. J. Clin. Invest. 69, 123-128.

CLEMENTS, G.B. and SUBAK-SHARPE, J.H. (1983). Recovery of herpes

simplex type 1 ts mutants from the dorsal root ganglia of mice. In: "Progress in Brain Research", Eds. P.O. Behan and V. ter Meulen, Amsterdam, Elsevier.

CLEMENTS, J.B., CORTINI, R. and WILKIE, N.M. (1976). Analysis of herpesvirus DNA substructure by means of restriction endonucleases. J. Gen. Virol. 30, 243-256.

CLEMENTS, J.B., MCLAUCHLAN, J. and MCGEOCH, D.J. (1979). Orientation of herpes simplex virus type 1 immediate early mRNAs. Nucl. Acids Res. 7, 77-91.

CLEMENTS, J.B., WATSON, R.J. and WILKIE, N.M. (1977). Temporal regulation of herpes simplex virus type 1 transcription: location of transcripts in the viral genome. Cell 12, 275-285.

COHEN, G.H. (1972). Ribonucleotide reductase activity of synchronised KB cells infected with herpes simplex virus. J. Virol. 9, 408-418.

COHEN, G.H., DIETZSCHOLD, B., PONCE DE LEON, M., LONG, D., GOLUB, E., VARRICHIO, A., PEREIRA, L. and EISENBERG, R.J. (1984). Localization and synthesis of an antigenic determinant of herpes simplex virus glycoprotein D that stimulates the production of neutralizing antibody. J. Virol. 49, 102-108.

COHEN, G.H., EISENBERG, R.J., PONCE DE LEON, M. and FRIEDMAN, H.M. (1985). Interaction of HSV-1 gC and human C3b: demonstration of binding between the purified components. Tenth International Herpesvirus Workshop, Ann Arbor, USA, p.158.

COHEN, G.H., HALLIBURTON, I.W. and EISENBERG, R.J. (1981). Glycoproteins of herpesviruses. In: "Human Herpesviruses", pp.549-554. Eds. A.J. Nahmias, W.R. Dowdle and R.F. Schinazi, New York: Elsevier Biomedical Press.

COHEN, G.H., KATZ, M., HYDREAN-STERN, C. and EISENBERG, R.J. (1978). Type common CP-1 antigen of herpes simplex virus is associated with a 59,000 molecular weight envelope glycoprotein. J. Virol. 27, 172-

COHEN, G.H., LONG, D. and EISENBERG, R.J. (1980). Synthesis and processing of glycoproteins gD and gC of herpes simplex virus type 1. J. Virol. 36, 429-439.

COHEN, G.H., PONCE DE LEON, M. and NICHOLS, C. (1972). Isolation of a herpes simplex virus-specific antigenic fraction which stimulates the production of neutralizing antibody. J. Virol. 10, 1,021-1,030.

COHEN, G.H., VAUGHAN, R.K. and LAWRENCE, W.C. (1971). Deoxyribonucleic acid synthesis in synchronized mammalian KB cells infected with herpes simplex virus. J. Virol. 7, 783-791.

COLEGATE, S.M., DORLING, P.R. and HUXTABLE, C.R. (1979). A spectrophotometric investigation of swainsonine, an alpha-mannosidase inhibitor isolated from Swainsona canescens. Aust. J. Chem. 32, 2,257-2,264.

COMPANS, R.W. and PINTER, A. (1975). Incorporation of sulphate into influenza virus glycoproteins. Virology 66, 151-160.

COMPTON, T. and COURTNEY, R.J. (1984a). Virus specific glycoproteins associated with the nuclear fraction of herpes simplex virus type 1-infected cells. J. Virol. 49, 594-597.

COMPTON, T. and COURTNEY, R.J. (1984b). Evidence for post-translational glycosylation of a non-glycosylated precursor protein of herpes simplex virus type 1. J. Virol. 52, 630-637.

CORDINGLEY, M.G., CAMPBELL, M.E. and PRESTON, C.M. (1983). Functional analysis of a herpes simplex type 1 promoter: identification of far-upstream regulatory sequences. Nucl. Acids Res. 11, 2,347-2,365.

COSTA, J., RABSON, A.S., YEE, C. and TRALKA, T.S. (1977). Immunoglobulin binding to herpes virus-induced Fc receptors inhibits virus growth. Nature 269, 251-252.

COSTA, J., YEE, C., NAKAMURA, Y. and RABSON, A. (1978). Characteristics of the Fc receptor induced by herpes simplex virus.

Intervirology 10, 32-39.

COSTA, J.C. and RABSON, A.S. (1975). Role of Fc receptors in herpes simplex virus infection. Lancet i, 77-78.

COSTA, R.H., DRAPER, K.G., BANKS, L., POWELL, K.L., COHEN, G., EISENBERG, R. and WAGNER, E.K. (1983). High-resolution characterisation of herpes simplex virus type 1 transcripts encoding alkaline exonuclease and a 50,000 dalton protein tentatively identified as a capsid protein. J. Virol. 48, 591-603.

COSTA, R.H., DRAPER, K.G., KELLY, T.J. and WAGNER, E.K. (1985). An unusual spliced herpes simplex virus type 1 transcript with sequence homology to Epstein-Barr virus DNA. J. Virol. 54, 317-328.

COURTNEY, R.J. (1976). Herpes simplex virus protein synthesis in the presence of 2-deoxy-D-glucose. Virology 73, 286-294.

CUMMINGS, R.D., TROWBRIDGE, I.S. and KORNFELD, S. (1982). A mouse lymphoma cell line resistant to the leukoagglutinating lectin from phaseolus vulgaris is deficient in UDP-Glc NAc: alpha-D-mannoside beta 1,6 N-acetylglucosaminyl-transferase. J. Biol. Chem. 257, 13,421-13,427.

DALES, S. and SILVERBERG, H. (1969). Viropexis of herpes simplex virus by HeLa cells. Virology 37, 475-480.

DALZIEL, R.G. (1984). Specific interactions between herpes simplex virus-induced proteins and DNA. Ph.D. Thesis. University of Glasgow.

DALZIEL, R.G. and MARSDEN, H.S. (1984). Identification of two herpes simplex virus type 1-induced proteins (21K and 22K) which interact specifically with the a sequence of herpes simplex virus DNA. J. Gen. Virol. 65, 1,467-1,475.

DARGAN, D. and SUBAK-SHARPE, J.H. (1983). Ultrastructure characterisation of herpes simplex virus type 1 (strain 17) temperature-sensitive mutants. J. Gen. Virol. 64, 1,311-1,326.

DATEMA, R., ROMERO, P.A., LEGLER, G. and SCHWARZ, R.T. (1982). Inhibition of formation of complex oligosaccharides by the glucosidase

inhibitor bromoconduritol. PNAS 79, 6,787-6,791.

DAVISON, A.J. (1981). Detailed structural aspects of the herpes simplex virus genome. Ph.D. Thesis, University of Glasgow.

DAVISON, A.J., MARSDEN, H.S. and WILKIE, N.M. (1981). One functional copy of the long terminal repeat gene specifying the immediate-early polypeptide (IE) suffices for a productive infection of human foetal lung cells by herpes simplex virus. J. Gen. Virol. 55, 179-191.

DAVISON, A.J. and SCOTT, J.E. DNA sequence of varicella-zoster virus. Manuscript in preparation.

DAVISON, A.J. and WILKIE, N.M. (1981). Nucleotide sequence of the joint between the L and S segments of herpes simplex types 1 and 2. J. Gen. Virol. 55, 315-331.

DAVISON, A.J. and WILKIE, N.M. (1983). Inversion of the two segments of the herpes simplex virus genome in intertypic recombinants. J. Gen. Virol. 64, 1-18.

DEBROY, C., PEDERSON, N. and PERSON, S. (1985). Nucleotide sequence of a herpes simplex virus type 1 gene that causes cell fusion. Virology 145, 36-48.

DELIUS, H. and CLEMENTS, J.B. (1976). A partial denaturation map of herpes simplex virus type 1 DNA: evidence for inversions of the unique DNA regions. J. Gen. Virol. 33, 125-133.

DE LUCA, N., BZIK, D., BOND, V.C., PERSON, S. and SNIPES, W. (1982). Nucleotide sequences of herpes simplex virus type 1 (HSV-1) affecting virus entry, cell fusion and production of glycoprotein gB (VP7). Virology 122, 411-423.

DE LUCA, N., BZIK, D., PERSON, S. and SNIPES, W. (1981). Early events in herpes simplex virus type 1 infection; photosensitivity of fluorescein isothiocyanate-treated virions. PNAS 78, 912-916.

DE LUCA, N., PERSON, S., BZIK, D.J. and SNIPES, W. (1984). Genome locations of temperature sensitive mutants in glycoprotein gB of herpes

simplex virus type 1. Virology 137, 382-389.

DERSE, D., BASTOW, K.F. and CHENG, Y.-C. (1982). Characterization of the DNA polymerases induced by a group of herpes simplex virus type 1 variants selected for growth in the presence of phosphonoformic acid. J. Biol. Chem. 257, 10,251-10,260.

DIERICH, M.P., LANDEN, B., SCHULZ, T. and FALKE, D. (1979). Protease activity on the surface of HSV-infected cells. J. Gen. Virol. 45, 241-244.

DIETZSCHOLD, B., EISENBERG, R.J., PONCE DE LEON, M., GOLUB, E., HUDECZ, F., VARRICHIO, A. and COHEN, G.H. (1984). Fine structure analysis of type-specific and type common antigenic sites of herpes simplex virus glycoprotein D. J. Virol. 52, 431-435.

DIX, R.D., PEREIRA, L. and BARINGER, J.R. (1981). Use of monoclonal antibodies directed against herpes simplex virus glycoproteins to protect mice against acute virus-induced neurological disease. Infect. Immun. 34, 192-199.

DIXON, R.A.F. and SCHAFFER, P.A. (1980). Fine-structure mapping and functional analysis of temperature-sensitive mutants in the gene encoding the herpes simplex virus type 1 immediate-early protein VP175. J. Virol. 36, 189-203.

DOCHERTY, J.J., MITCHELL, W.R. and THOMSON, C.J. (1973). Abortive herpes simplex virus replication in rous sarcoma virus-transformed cells (37665). Proc. Soc. Exp. Biol. Med. 144, 697-704.

DOCHERTY, J.J., SUBAK-SHARPE, J.H. and PRESTON, C.M. (1981). Identification of a virus-specific polypeptide associated with a transforming fragment (BglII N) of herpes simplex virus type 2 DNA. J. Virol. 40, 126-132.

DOLYNIUK, M., PRITCHETT, R. and KIEFF, E. (1976). Proteins of Epstein-Barr virus. I. Analysis of the polypeptides of purified enveloped Epstein-Barr virus. J. Virol. 17, 935-949.

DOWBENKO, D.J. and LASKY, L.A. (1984). Extensive homology between the herpes simplex virus type 2 glycoprotein F gene and the herpes simplex virus type 1 glycoprotein C gene. J. Virol. 52, 154-163.

DRAPER, K.G., COSTA, R.H., LEE, G.T.-Y., SPEAR, P.G. and WAGNER, E.K. (1984a). Molecular basis of the glycoprotein-C-negative phenotype of herpes simplex virus type 1 macroplaque strain. J. Virol. 51, 578-585.

DRAPER, K.G., FRINK, R., DEVI, G.B., SWAIN, M., GALLOWAY, D. and WAGNER, E.K. (1984b). Herpes simplex virus types 1 and 2 homology in the region between 0.58 and 0.68 map units. J. Virol. 52, 615-623.

DULBECCO, R. and VOGT, M. (1954). Plaque formation and isolation of pure lines with polio myelitis. J. Exp. Med. 99, 167-182.

DUNPHY, G.W. and ROTHMAN, J.E. (1983). Compartmentation of asparagine-linked oligosaccharide processing in the golgi apparatus. J. Cell Biol. 97, 270-275.

DUTIA, B.M. (1983). Ribonucleotide reductase induced by herpes simplex virus has a virus-specified constituent. J. Gen. Virol. 64, 513-521.

EBERLE, R. and COURTNEY, R.J. (1980a). Preparation and characterization of specific antisera to individual glycoprotein antigens comprising the major glycoprotein region of herpes simplex virus type 1. J. Virol. 35, 902-917.

EBERLE, R. and COURTNEY, R.J. (1980b). gA and gB glycoproteins of herpes simplex virus type 1: two forms of a single polypeptide. J. Virol. 36, 665-675.

EFSTRATIADIS, A., POSAKONY, J.W., MANIATIS, T., LAWN, R.M., O'CONNELL, C., SPRITZ, R.A., DE RIEL, J.K., FORGET, B.G., WEISSMAN, S.M., SLIGHTOM, J.L., BLECHL, A.E., SMITHIES, O., BARALLE, F.E., SHOULDERS, C.C. and PROUDFOOT, N.J. (1980). The structure and evolution of the human beta-globin gene family. Cell 21, 653-668.

EISENBERG, R.J., HYDREAN-STERN, C. and COHEN, G.H. (1979). Structural analysis of precursor and product forms of type-common

envelope glycoprotein D (CP-1 antigen) of herpes simplex virus type 1. J. Virol. 31, 608-620.

EISENBERG, R.J., LONG, D., HOGUE-ANGELETTI, R. and COHEN, G.H. (1984). Amino-terminal sequence of glycoprotein D of herpes simplex virus types 1 and 2. J. Virol. 49, 265-268.

EISENBERG, R.J., LONG, D., PEREIRA, L., HAMPAR, B., ZWEIG, M. and COHEN, G.H. (1982a). Effect of monoclonal antibodies on limited proteolysis of native glycoprotein gD of herpes simplex virus type 1. J. Virol. 41, 478-488.

EISENBERG, R.J., LONG, D., PONCE DE LEON, M., MATTHEWS, J.T., SPEAR, P.G., GIBSON, M.G., LASKY, L.A., BERMAN, P., GOLUB, E. and COHEN, G.H. (1985. Localization of epitopes of herpes simplex virus type 1 glycoprotein D. J. Virol. 53, 634-644.

EISENBERG, R.J., PONCE DE LEON, M. and COHEN, G.H. (1980). Comparative structural analysis of glycoprotein gD of herpes simplex virus types 1 and 2. J. Virol. 35, 428-435.

EISENBERG, R.J., PONCE DE LEON, M., PEREIRA, L., LONG, D. and COHEN, G.H. (1982b). Purification of glycoprotein gD of herpes simplex virus types 1 and 2 by use of monoclonal antibody. J. Virol. 41, 1,099-1,104.

EJERCITO, P.M., KIEFF, E.D. and ROIZMAN, B. (1968). Characterization of herpes simplex virus strains differing in their effects on social behaviour of infected cells. J. Gen. Virol. 2, 357-364.

ELBEIN, A.D., SOLF, R., DORLING, P.R. and VOSBECK, K. (1981). Swainsonine: an inhibitor of glycoprotein processing. PNAS 78, 7,393-7,397.

EMR, S., HALL, M.N. and SILHAVY, T.J. (1980). A mechanism of protein localisation: the signal hypothesis and bacteria. J. Cell Biol. 86, 701-711.

ENDERS, J.F. and PEEBLES, T.C. (1954). Propagation in tissue cultures of cytopathogenic agents from patients with measles. Proc. Soc. Exp. Biol.

Med. 86, 277-286.

ENGELMAN, D.M. and STEITZ, T.A. (1981). The spontaneous insertion of proteins into and across membranes: the helical hairpin hypothesis. Cell 23, 411-422.

EPSTEIN, A.L. and JACQUEMONT, B. (1983). Virus polypeptide synthesis induced by herpes simplex virus type 1. Virology 115, 149-160.

EPSTEIN, A.L., JACQUEMONT, B. and MACHUCA, I. (1984). Infection of a restrictive cell line (XC) cells by intratypic recombinants of HSV-1: relationship between penetration of the virus and relative amounts of glycoprotein C. Virology 132, 315-324.

EPSTEIN, M.A. (1962). Observations on the structure of mature herpes simplex virus and on the composition of the nucleoid. J. Exp. Med. 115, 1-11.

EPSTEIN, M.A., HENLE, W., ACHONG, B.G. and BARR, Y.M. (1965). Morphological and biological studies on a virus in cultured lymphoblasts from Burkitt's lymphoma. J. Exp. Med. 121, 761-770.

ERICKSON, J.S. and KAPLAN, A.S. (1973). Synthesis of proteins in cells infected with herpesvirus. IX. Sulphated proteins. Virology 55, 94-102.

ETCHISON, J.R. and HOLLAND, J.J. (1974). Carbohydrate composition of the membrane glycoprotein of vesicular stomatitis virus grown in four mammalian cell lines. PNAS 71, 4,011-4,014.

EVERETT, R.D. (1983). DNA sequence elements required for regulated expression of the HSV-1 glycoprotein D gene lie within 83bp of the RNA capsites. Nucl. Acids Res. 11, 6,647-6,666.

EVERETT, R.D. (1984a). A detailed analysis of an HSV-1 early promoter: sequences involved in transactivation by viral immediate early gene products are not early-gene specific. Nucl. Acids Res. 12, 3,037-3,056.

EVERETT, R.D. (1984b). Trans activation of transcription by herpes virus products: requirement for two HSV-1 immediate-early polypeptides for maximum activity. The EMBO Journal 3, 3,135-3,141.

EVERETT, R.D. (1985). Activation of cellular promoters during herpes virus infection of biochemically transformed cells. The EMBO Journal 4, 1,973-1,980.

EVERETT, R.D. and DUNLOP, M. (1984). Trans activation of plasmid-borne promoters by adenovirus and several herpes group viruses. Nucl. Acids Res. 12, 5,969-5,978.

EYLAR, E.R. (1965). On the biological role of glycoproteins. J. Theor. Biol. 10, 89-113.

FALKE, D., SIEGERT, R. and VOGEL, W. (1959). Elektronenmikroskopische befunde zur frage der doppel membranbildung des herpes simplex virus. Arch. ges. Virusforsch 9, 484-496.

FARNHAM, A.E. and NEWTON, A.A. (1959). The effect of some environmental factors on herpes simplex virus grown in HeLa cells. Virology 7, 449-461.

FARQUAR, M.G. and PALADE, G.E. (1981). The golgi apparatus (complex) - (1954-1981) - from artefact to centre stage. J. Cell Biol. 91, 77s-103s

FEORINO, P.M., SHORE, S.L. and REIMER, C.B. (1977). Detection by indirect immunofluorescence of Fc receptors in cells acutely infected with herpes simplex virus. Int. Arch. Allergy Appl. Immunol. 53, 222-233.

FEARON, D.T. (1979). Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. PNAS 76, 5,867-5,871.

FENWICK, M., MORSE, L.S. and ROIZMAN, B. (1979). Anatomy of herpes simplex virus DNA. XI. Apparent clustering of functions effecting rapid inhibition of host DNA and protein synthesis. J. Virol. 29, 825-827.

FENWICK, M.L. and WALKER, M.J. (1978). Suppression of the synthesis of cellular macromolecules by herpes simplex virus. J. Gen. Virol. 41, 37-51.

FENWICK, M.L., WALKER, M.J. and MARSHALL, L. (1980). Some

characteristics of an early protein (ICP22) synthesised in cells infected with herpes simplex virus. J. Gen. Virol. 47, 333-341.

FINNE, J., KRUSIUS, T. and RAUVALA, H. (1977). Occurrence of disialosyl groups in glycoproteins. Biochem. Biophys. Res. Commun. 74, 405-410.

FISHER, F.B. and PRESTON, V.G. (1986). Isolation and characterisation of herpes simplex virus type 1 mutants which fail to induce dUTPase activity. Virology 149, 190-197.

FLEISCHER, B., FLEISCHER, S. and OZAWA, H. (1969). Isolation and characterization of golgi membranes from bovine liver. J. Cell Biol. 43, 59-79.

FRAME, M.C., MCGEOCH, D.J., RIXON, F.J. and ORR, A.C. The 10K virion phosphoprotein encoded by US gene 9 from herpes simplex virus type 1. (Submitted for publication, Virology).

FRAME, M.C., MARSDEN, H.S. and DUTIA, B.M. (1985). The ribonucleotide reductase induced by herpes simplex virus type 1 involves minimally a complex of two polypeptides (136K and 38K). J. Gen. Virol. 66, 1,581-1,587.

FRAME, M.C., MARSDEN, H.S. and MCGEOCH, D.J. Novel herpes simplex virus type 1 glycoproteins reactive with an antiserum against an oligopeptide from part of the sequence of the gene US4 product. In Press. J. Gen. Virol.

FRANCKE, B., MOSS, H., TIMBURY, M.C. and HAY, J. (1978). Alkaline DNase activity in cells infected with a temperature-sensitive mutant of herpes simplex virus type 2. J. Virol. 26, 209-213.

FREEMAN, M.J. and POWELL, K.L. (1982). DNA-binding properties of a herpes simplex virus immediate early protein. J. Virol. 44, 1,084-1,087.

FRENKEL, N. and ROIZMAN, B. (1972). Ribonucleic acid synthesis in cells infected with herpes simplex virus: controls of transcription and abundance. PNAS 69, 2,654-2,658.

FRENKEL, N., SILVERSTEIN, S., CASSAI, E. and ROIZMAN, B. (1973). RNA synthesis in cells infected with herpes simplex virus. VII. Control of transcription and of transcript abundancies of unique and common sequences of herpes simplex virus 1 and 2. J. Virol. 11, 886-892.

FRIEDMAN, A., COWARD, J.E., ROSENKRANZ, H.S. and MORGAN, C. (1975). Electron microscopic studies on assembly of herpes simplex virus upon removal of hydroxyurea block. J. Gen. Virol. 26, 171-181.

FRIEDMAN, A., SCHLOMAI, J. and BECKER, Y. (1977). Electron microscopy of herpes simplex virus DNA molecules isolated from infected cells by centrifugation in CsCl density gradients. J. Virol. 34, 507-522.

FRIEDMAN, H.M., COHEN, G.H., EISENBERG, R.J., SEIDEL, C.A. and CINES, D.B. (1984). Glycoprotein C of herpes simplex virus 1 acts as a receptor for the C3b complement component on infected cells. Nature 309, 633-635.

FRINK, R.J., ANDERSON, K.P. and WAGNER, E.K. (1981). Herpes simplex virus type 1 HindIII fragment encodes spliced and complementary mRNA species. J. Virol. 39, 559-572.

FRINK, R.J., EISENBERG, R., COHEN, G. and WAGNER, E.K. (1983). Detailed analysis of the portion of the herpes simplex virus type 1 genome encoding glycoprotein C. J. Virol. 45, 634-647.

FURLONG, D., SWIFT, H. and ROIZMAN, B. (1972). Arrangement of herpesvirus deoxyribonucleic acid in the core. J. Virol. 10, 1,071-1,074.

GAHMBERG, C.G., JOHINEN, M., KARHI, K.K. and ANDERSSON, L.C. (1980). Effect of tunicamycin on the biosynthesis of the major human red cell sialoglycoprotein, glycophorin A, in the leukaemia cell line K562. J. Biol. Chem. 255, 2,169-2,175.

GARFINKLE, B. and MCAUSLAN, B.R. (1973). Non-cytopathic, non-productive infection by herpes simplex viruses types 1 and 2. Intervirology 1, 362-365.

GELLERT, M. (1981). DNA topoisomerases. Ann. Rev. Biochem. 50, 879-

910.

GHANDI, S.S., STANLEY, P., TAYLOR, J.M. and WHITE, D.O. (1972). Inhibition of influenza virus glycoprotein synthesis by sugars. Microbios. 5, 41-50.

GIBBS, J., CHIOU, H., HALL, J., KELLY, R., MOUNT, D., RETONDO, M., WELLER, S. and COEN, D. (1985). Sequence and mapping studies of the herpes simplex virus DNA polymerase locus predict a large C-terminal substrate binding domain. Tenth International Herpesvirus Workshop, Ann Arbor, USA, p.102.

GIBSON, M.G. and SPEAR, P.G. (1983). Insertion mutants of herpes simplex virus have a duplication of the glycoprotein D gene and express two different forms of glycoprotein D. J. Virol. 48, 396-404.

GIBSON, R., LEAVITT, R., KORNFELD, S. and SCHLESINGER, S. (1978). Synthesis and infectivity of vesicular stomatitis virus containing non-glycosylated G protein. Cell 13, 671-679.

GIBSON, R., SCHLESINGER, S. and KORNFELD, S. (1979). The non-glycosylated glycoprotein of vesicular stomatitis virus is temperature-sensitive and undergoes intracellular aggregation at elevated temperatures. J. Biol. Chem. 254, 3,600-3,607.

GIBSON, W. and ROIZMAN, B. (1971). Compartmentalization of spermine and spermidine in the herpes simplex virion. PNAS 68, 2,818-2,821.

GIBSON, W. and ROIZMAN, B. (1972). Proteins specified by herpes simplex virus. VIII. Characterization and composition of multiple capsid forms of subtypes 1 and 2. J. Virol. 10, 1,044-1,052.

GIBSON, W. and ROIZMAN, B. (1974). Proteins specified by herpes simplex virus. X. Staining and radiolabelling properties of B capsid and virion proteins in polyacrylamide gels. J. Virol. 13, 155-165.

GILMORE, R., BLOBEL, G. and WALTER, P. (1982a). Protein translocation across the endoplasmic reticulum. I. Detection in the microsomal membrane of a receptor for the signal recognition particle. J.

Cell Biol. 95, 463-469.

GILMORE, R., WALTER, P. and BLOBEL, G. (1982b). Protein translocation across the endoplasmic reticulum. II. Isolation and characterisation of the signal recognition particle receptor. J. Cell Biol. 95, 470-477.

GLORIOSO, J., SYCZESIUL, M.S., MARLIN, S.D. and LEVINE, M. (1983). Inhibition of glycosylation of herpes simplex virus glycoproteins: immunogenicity and antigenicity of partially glycosylated glycoproteins. Virology 126, 1-18.

GODOWSKI, P.J. and KNIPE, D.M. (1983). Mutations in the major DNA-binding protein gene of herpes simplex virus type 1 result in increased levels of viral gene expression. J. Virol. 47, 478-486.

GODOWSKI, P.J. and KNIPE, D.M. (1985). Identification of a herpes simplex virus function that represses late gene expression from parental viral genomes. J. Virol. 55, 357-365.

GOLDBERG, D.E. and KORNFELD, S. (1983). Evidence for extensive subcellular organisation of asparagine-linked oligosaccharide processing and lysosomal enzyme phosphorylation. J. Biol. Chem. 258, 3,159-3,165.

GOLDSTEIN, I.J. and HAYES, C.E. (1978). The lectins: carbohydrate binding properties of plants and animals. Adv. Carbohydr. Chem. Biochem. 35, 127-350.

GOODHEART, C.R., PLUMMER, G. and WANER, J.L. (1968). Density differences of DNA of human herpes simplex viruses, types 1 and 2. Virology 35, 473-475.

GOTTLIEB, C., KORNFELD, S. and SCHLESINGER, S. (1979). Restricted replication of two alphaviruses in ricin-resistant mouse L cells with altered glycosyltransferase activities. J. Virol. 29, 344-351.

GRAFSTROM, R.H., ALWINE, J.C., STEINHART, W.L. and HILL, C.W. (1974). Terminal repetitions in herpes simplex virus type 1 DNA. Cold Spring Harbor Symp. Quant. Biol. 39, 679-683.

GRAFSTROM, R.H., ALWINE, J.C., STEINHART, W.L., HILL, C.W. and HYMAN, R.W. (1975). The terminal repetitions of herpes simplex virus DNA. Virology 67, 144-157.

GRAHAM, F.L., VELDHUISEN, G. and WILKIE, N.M. (1973). Infectious herpesvirus DNA. Nature New Biol. 245, 265-266.

GRAY, C.P. and KAERNER, H.C. (1984). Sequence of the putative origin of replication in the U_L region of herpes simplex virus type 1 ANG DNA. J. Gen. Virol. 65, 2,109-2,119.

GREEN, M.T., COURTNEY, R.J. and DUNKEL, E.G. (1981b). Detection of an immediate early herpes simplex virus type 1 polypeptide in trigeminal ganglia from latently infected animals. Infect. Immun. 34, 987-992.

GREEN, M.T., ROSBOROUGH, J.P. and JONES, D.B. (1981a). In vivo reactivation of herpes simplex virus in rabbit trigeminal ganglia: electrode model. Infect. Immun. 34, 69-74.

GRIFFITHS, G., QUINN, P. and WARREN, G. (1983). Dissection of the golgi complex. I. Monensin inhibits the transport of viral membrane proteins from medial to trans golgi cisternae in baby hamster kidney cells infected with Semliki Forest Virus. J. Cell Biol. 96, 835-850.

GRINNA, L.S. and ROBBINS, P.W. (1979). Glycoprotein biosynthesis. J. Biol. Chem. 254, 8,814-8,818.

GROSS, V., ANDUS, T., TRAN-THI, T.A., SCHWARZ, R.T., DECKER, K. and HEINRICH, P.C. (1983). 1-deoxynojirimycin impairs oligosaccharide processing of alpha 1-proteinase inhibitor and inhibits its secretion in primary cultures of rat hepatocytes. J. Biol. Chem. 258, 12,203-12,209.

GRUTER, W. (1924). Das herpesvirus seine aetiologische und klinische bedeutung. Muenich. Med. Wochenschr. 71, 1,058-1,060.

GUSTAFSOHN, D.P. (1970). Pseudorabies in: "Diseases of Swine", 3rd edition. pp.337-355. Ed. H.W. Dunne, Iowa State University Press.

HAARR, L. and MARSDEN, H.S. (1981). Two dimensional gel analysis of

HSV type-1 induced polypeptides and glycoprotein processing. J. Gen. Virol. 52, 77-92.

HAARR, L., MARSDEN, H.S., PRESTON, C.M., SMILEY, J.R., SUMMERS, W.C. and SUMMERS, W.P. (1985). Utilisation of internal AUG codons for initiation of protein synthesis directed by messenger RNAs from normal and mutant genes encoding herpes simplex virus thymidine kinase. J. Virol. 56, 512-519.

HAGOPIAN, A. and EYLAR, E.H. (1968). Glycoprotein biosynthesis: studies on the receptor specificity of the polypeptidyl: N-acetylgalactosaminyl transferase from bovine submaxillary glands. Arch. Biochem. Biophys. 128, 422-433.

HALLIBURTON, I.W. (1972). Biological comparisons of type 1 and type 2 herpes simplex viruses. In: "Oncogenesis and Herpesviruses". pp.432-438. Eds. P.M. Biggs, G. deThe, and L.N. Payne. IARC Scientific Publications, No. 2, Lyon.

HALLIBURTON, I.W. (1980). Intertypic recombinants of herpes simplex virus. J. Gen. Virol. 48, 1-23.

HALLIBURTON, I.W., MORSE, L.S., ROIZMAN, B. and QUINN, K.E. (1980). Mapping of the thymidine kinase genes of type 1 and type 2 herpes simplex viruses using intertypic recombinants. J. Gen. Virol. 49, 235-253.

HAMBLIN, J. and KENT, S.P. (1973). Possible role of phytohaemagglutinin in Phaseolus vulgaris L. Nature New Biol. 245, 28-30.

HAMMARSTROM, S., MURPHY, L.A., GOLDSTEIN, I,J. and ETYLER, M.E. (1977). Carbohydrate binding specificity of four N-acetyl-D-galactosamine-"specific" lectins: helix pomatia A haemagglutinin, soybean agglutinin, lima bean agglutinin and dolichos biflorus lectin. Biochemistry 16, 2,750-2,755.

HANOVER, J., LENNARZ, W.J. and YOUNG, J.D. (1980). Synthesis of N- and O-linked glycopeptides in oviduct membrane preparations. J. Biol. Chem. 255, 6,713-6,716.

HARPAZ, N. and SCHACHTER, H. (1980a). Control of glycoprotein synthesis. Bovine colostrum UDP-N-acetylglucosamine: alpha-D-mannoside beta 2-acetylglucosaminyl-transferase I separation from UDP-N-acetyl-glucosaminyl: alpha-D-mannoside beta 2-N-acetylglucosaminyl-transferase II, partial purification and substrate specificity. J. Biol. Chem. 255, 4,885-4,893.

HARPAZ, N. and SCHACHTER, H. (1980b). Control of glycoprotein synthesis. Processing of asparagine-linked oligosaccharides by one or more rat liver golgi alpha-D-mannosidases dependent on the prior action of UDP-N-acetylglucosamine: alpha-D-mannoside beta 2-N-acetylglucosaminyl-transferase I. J. Biol. Chem. 255, 4,894-4,902.

HAY, J. and SUBAK-SHARPE, J.H. (1976). Mutants of herpes simplex virus types 1 and 2 that are resistant to phosphonoacetic acid induce altered DNA polymerase activities in infected cells. J. Gen. Virol. 31, 145-148.

HAY, J., PEREIRA, P., GENTRY, G.A. and SUBAK-SHARPE, J.H. (1971). Herpesvirus specified proteins. In: "Strategy of the viral genome", pp.355-376. Eds. G.E. Wolstenholme and M. O'Connor. Ciba Foundation Symp. Churchill Livingstone, London.

HAY, R.T. and HAY, J. (1980). Properties of herpesvirus-induced "immediate early" polypeptides. Virology 104, 230-234.

HAYWARD, G.S., FRENKEL, N. and ROIZMAN, B. (1975a). The anatomy of herpes simplex virus DNA: strain difference and heterogeneity in the location of restriction endonuclease sites. PNAS 72, 1,768-1,772.

HAYWARD, G.S., JACOB, R.J., WADSWORTH, S.C. and ROIZMAN, B. (1975b). Anatomy of herpes simplex virus DNA: evidence for four populations that differ in the relative orientations of their long and short components. PNAS 72, 4,243-4,247.

HEINE, J.W., HONESS, R.W., CASSAI, E. and ROIZMAN, B. (1974). Proteins specified by herpes simplex virus. XII. The virion polypeptides of

type 1 strains. J. Virol. 14, 640-651.

HEINE, J.W., SPEAR, P.G. and ROIZMAN, B. (1972). Proteins specified by herpes simplex virus. VI. Viral proteins in the plasma membrane. J. Virol. 9, 431-439.

HEINE, V. and COTTLER-FOX, M. (1975). Electron microscopic observations on the composition of herpes-type viruses. In: "Oncogenesis and Herpesviruses", II, part 1, pp.103-110. Eds. G. deThe, M.A. Epstein and H. zur Hausen. IARC Scientific Publications, No. 11, Lyon.

HENDERSON, L.E., OROSZLAN, S. and KONIGSBERG, W. (1979). A micromethod for complete removal of dodecyl sulphate from proteins by ion-pair extraction. Analytical Biochem. 93, 153-157.

HENNING, R. and LANGE-MUTSCHLER, J. (1983). Tightly associated lipids may anchor SV40 large T antigen in plasma membrane. Nature 305, 736-738.

HILL, T.J. (1985). Herpes simplex virus latency. In: "the Herpesviruses", volume 3. pp.175-240. Ed. B. Roizman. Plenum Press, New York.

HIRS, C.A.W. (1967). Performic acid oxidation. In: "Methods in Enzymology", volume 11, pp.189-199. Eds. S.P. Colowick and N.O. Kaplan. Academic Press, New York.

HIRSCH, I., CABRAL, G., PATTERSON, M. and BISWAL, N. (1977). Studies on the intracellular replicating DNA of herpes simplex virus type 1. Virology 81, 48-61.

HOCHBERG, E. and BECKER, Y. (1968). Adsorption, penetration and uncoating of herpes simplex virus. J. Gen. Virol. 2, 231-241.

HOFFMAN, P.J. and CHENG, Y. (1978). The deoxyribonuclease induced after infection of KB cells by herpes simplex virus type 1 or type 2. J. Biol. Chem. 253, 3,557-3,562.

HOFFMAN, P.J. and CHENG, Y. (1979). DNase induced after infection of KB cells by herpes simplex virus type 1 or type 2. J. Virol. 32, 449-457.

HOGGAN, M.D. and ROIZMAN, B. (1959). The isolation and properties of a variant of herpes simplex producing multinucleated giant cells in monolayer cultures in the presence of antibody. American J. Hyg. 70, 208-219.

HOLLAND, L.E., ANDERSON, K.P., SHIPMAN, C. and WAGNER, E.K. (1980). Viral DNA synthesis is required for the efficient expression of specific herpes simplex virus type 1 mRNA species. Virology 101, 10-24.

HOLLAND, L.E., ANDERSON, K.P., STRINGER, J.R. and WAGNER, E.K. (1979). Isolation and localization of herpes simplex virus type 1 mRNA abundant before viral DNA synthesis. J. Virol. 31, 447-462.

HOLLAND, L.E., SANDRI-GOLDIN, R.M., GOLDIN, A.L., GLORIOSO, J.C. and LEVINE, M. (1984a). Transcriptional and genetic analysis of the herpes simplex virus type 1 genome: co-ordinates 0.29-0.45. J. Virol. 49, 947-959.

HOLLAND, T.C., HOMA, F.L., MARLIN, S.D., LEVINE, M. and GLORIOSO, J. (1984b). Herpes simplex virus type 1 glycoprotein C-negative mutants exhibit multiple phenotypes, including secretion of truncated glycoproteins. J. Virol. 52, 566-574.

HOLLAND, T.C., MARLIN, S.D., LEVINE, M. and GLORIOSO, J.C. (1983a). Antigenic variants of herpes simplex virus selected with glycoprotein-specific monoclonal antibodies. J. Virol. 45, 672-682.

HOLLAND, T.C., SANDRI-GOLDIN, R.M., HOLLAND, L.E., MARLIN, S.D., LEVINE, M. and GLORIOSO, J.C. (1983b). Physical mapping of the mutation in an antigenic variant of herpes simplex virus type 1 by use of an immunoreactive plaque assay. J. Virol. 46, 649-652.

HOLMES, I.H. and WATSON, D.H. (1963). An electron microscope study of the attachment and penetration of herpes virus in BHK21 cells. Virology 21, 112-123.

HONESS, R.W., BUCHAN, A., HALLIBURTON, I.W. and WATSON, D.H. (1980). Recombination and linkage between structural and regulatory genes

of herpes simplex virus type 1: study of the functional organization of the genome. J. Virol. 34, 716-742.

HONESS, R.W. and ROIZMAN, B. (1973). Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and non-structural herpes virus polypeptides in the infected cell. J. Virol. 12, 1,347-1,365.

HONESS, R.W. and ROIZMAN, B. (1974). Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. 14, 8-19.

HONESS, R.W. and ROIZMAN, B. (1975). Proteins specified by herpes simplex virus. XIII. Glycosylation of viral glycoproteins. J. Virol. 16, 1,308-1,326.

HONESS, R.W. and WATSON, D.H. (1974). Herpes simplex virus-specific polypeptides studied by polyacrylamide gel electrophoresis of immunne precipitates. J. Gen. Virol. 22, 171-185.

HONESS, R.W. and WATSON, D.H. (1977). Herpes simplex virus resistance and sensitivity to phosphonoacetic acid. J. Virol. 21, 584-600.

HOPE, R.G. and MARSDEN, H.S. (1983). Processing of glycoproteins induced by herpes simplex virus type 1: sulphation and nature of the oligosaccharide linkages. J. Gen. Virol. 64, 1,943-1,953.

HOPE, R.G., PALFREYMAN, J.W., SUH, M. and MARSDEN, H.S. (1982). Sulphated glycoproteins induced by herpes simplex virus. J. Gen. Virol. 58, 399-415.

HOWARD, D.J., HAY, J., MELVIN, W.T. and DURHAM, J.P. (1974). Changes in DNA and RNA synthesis and associated enzyme activities after the stimulation of serum-depleted BHK21/C13 cells by the addition of serum. Expt. Cell Res. 86, 31-42.

HUANG, A.S. and WAGNER, R.R. (1964). Penetration of herpes simplex virus into human epidermoid cells. Proc. Soc. Exp. Biol. Med. 116, 863-869.

HUANG, C.C. and AMINOFF, D. (1972). Enzymes that destroy blood group specificity. V. The oligosaccharase of Clostridium perfringens. J. Biol. Chem. 247, 6,737-6,742.

HUBBARD, A.L, WILSON, G., ASHWELL, G. and STUKENBROK, H. (1979). An electron microscope autoradiographic study of the carbohyrate recognition systems in rat liver. I. Distribution of ^{125}I-ligands among the rat liver cell types. J. Cell Biol. 83, 47-64.

HUBBARD, S.C. and IVATT, R.J. (1981). Synthesis and processing of asparagine-linked oligosaccharides. Ann. Rev. Biochem. 50, 555-583.

HUBBARD, S.C. and ROBBINS, P.W. (1979). Synthesis and processing of protein-linked oligosaccharides in vivo. J. Biol. Chem. 254, 4,568-4,576.

HUMMELAR, K., TOMASSINI, N. and ZAJAC, B. (1969). Early events in herpes simplex virus infection: a radioautographic study. J. Virol. 4, 67-74.

HUSZAR, D. and BACCHETTI, S. (1981). Partial purification and characterization of the ribonucleotide reductase induced by herpes simplex virus infection of mammalian cells. J. Virol. 37, 580-588.

HUSZAR, D., BEHARRY, S. and BACCHETTI, S. (1983). Herpes simplex virus-induced ribonucleotide reductase: development of antibodies specific for the enzyme. J. Gen. Virol. 64, 1,327-1,335.

HUTTNER, W.B. (1982). Sulphation of tyrosine residues - a widespread modification of proteins. Nature 299, 273-276.

HUTTNER, W.B. (1984). Determination and occurrence of tyrosine-O-sulphate in proteins. In: "Post-translational modifications. Methods in Enzymology", volume 107, pp.200-223. Eds. K. Moldave and F. Wold.

IKURA, K., BETZ, J.L., SADLER, J.R. and PIZER, L.I. (1983). RNAs transcribed from a 3.6-kilobase SmaI fragment of the short unique region of the herpes simplex virus type 1 genome. J. Virol. 48, 460-471.

INOUYE, M. and HALEGOUA, S. (1980). Secretion and membrane localisation of proteins in Escherichia coli. Crit. Rev. Biochem. 7, 339-

371.

ISHAK, R., ANDIMAN, W.A. and TUCKER, G. (1984). Absence of IgG Fc-receptors on varicella-zoster virus-infected cells. J. Med. Virol. 13, 261-267.

ISOM, H.C., LIAO, W.S.L., TAYLOR, J.M., WILLWERTH, G.E. and EADLINE, T.S. (1983). Rapid and selective shut off of plasma protein production in herpes simplex virus type 2-infected hepatoma cells. Virology 126, 548-562.

ITO, S., YAMASHITA, K., SPIRO, R.G. and KOBATA, A. (1977). Structure of a carbohydrate moiety of a unit A glycopeptide of calf thyroglobulin. J. Biochem. (Tokyo) 81, 1,621-1,631.

ITO, T., TAKATSUKA, A., KAWAMURA, K., SATO, K. and TAMURA, G. (1980). Isolation and structure of components of tunicamycin. Agric. Biol. Chem. 44, 695-698.

IWASAKA, T., ODA, H., MORI, R., MIYAZONO, J. and NAGAFUCHI, S. (1979). Production of tubular structures in Vero cells infected with herpes simplex virus type 2: effect of ultraviolet light irradiation and antimetabolites. J. Gen. Virol. 44, 57-67.

JACKSON, R.C. and BLOBEL, G. (1977). Post-translational cleavage of presecretory proteins with an extract of rough microsomes from dog pancreas containing signal peptidase activity. PNAS 74, 5,598-5,602.

JACOB, R.J., MORSE, L.S. and ROIZMAN, B. (1979). Anatomy of herpes simplex virus DNA. XII. Accumulation of head to tail concatamers in nuclei of infected cells and their role in the generation of the four isomeric arrangements of viral DNA. J. Virol. 29, 448-457.

JACOB, R.J. and ROIZMAN, B. (1977). Anatomy of herpes simplex virus DNA. VIII. Properties of the replicating DNA. J. Virol. 22, 394-411.

JAMIESON, A.T., GENTRY, G.A. and SUBAK-SHARPE, J.H. (1974). Induction of both thymidine kinase and deoxycytidine kinase activity by herpes simplex viruses. J. Gen. Virol. 24, 465-480.

JAMIESON, A.T. and SUBAK-SHARPE, J.H. (1974). Biochemical studies on the herpes simplex virus-specified deoxypyrimidine kinase activity. J. Gen. Virol. 24, 481-492.

JAMIESON, J.C. (1977). Studies on the site of addition of sialic acid and glucosamine to rat alpha$_1$-acid glycoprotein. Can. J. Biochem. 55, 408-414.

JEAN, J.-H. and BEN-PORAT, T. (1976). Appearance in vivo of single stranded complementary ends on parental herpesvirus DNA. PNAS 73, 2,674-2,678.

JEAN, J.-H., BLANKENSHIP, M.L. and BEN-PORAT, T. (1977). Replication of herpesvirus DNA. I. Electron microscopic analysis of replicative structures. Virology 79, 281-291.

JOHANSSON, P.J.H., HALLBERG, T., OXELIUS, V.-A., GRUBB, A. and BLOMBERG, J. (1984). Human immunoglobulin class and subclass specificity of Fc receptors induced by herpes simplex virus type 1. J. Virol. 50, 796-804.

JOHANSSON, P.J.H., MYHRE, E.B. and BLOMBERG, J. (1985). Specificity of Fc-receptors induced by herpes simplex virus type 1: comparison of immunoglobulin G from different animal species. J. Virol. 56, 489-494.

JOHNSON, D.C. and SCHLESINGER, M.J. (1980). Vesicular Stomatitis Virus and Sindbis Virus glycoprotein transport to the cell surface is inhibited by ionophores. Virology 103, 407-424.

JOHNSON, D.C. and SPEAR, P.G. (1982). Monensin inhibits the processing of herpes simplex virus glycoproteins, their transport to the cell surface, and the egress of virions from infected cells. J. Virol. 43, 1,102-1,112.

JOHNSON, D.C. and SPEAR, P.G. (1983). O-linked oligosaccharides are acquired by herpes simplex virus glycoproteins in the golgi apparatus. Cell 32, 987-997.

JOHNSON, D.C. and SPEAR, P.G. (1984). Evidence for translational regulation of herpes simplex virus type 1 gD expression. J. Virol. 51, 389-394.

JOHNSON, D.C., WITTELS, M. and SPEAR, P.G. (1984). Binding to cells of virosomes containing herpes simplex virus type 1 glycoproteins and evidence for fusion. J. Virol. 52, 238-247.

JONES, P.C. and ROIZMAN, B. (1979). Regulation of herpes virus macromolecular synthesis. VIII. The transcription programme consists of three phases during which both the extent and accumulation of RNA in the cytoplasm are regulated. J. Virol. 31, 299-314.

KALUZA, G., SCHMIDT, M.F.G. and SCHOLTISSEK, C. (1973). Effect of 2-deoxy-D-glucose on the multiplication of Semliki Forest Virus and the reversal of the block by mannose. Virology 54, 179-189.

KALUZA, G., SCHOLTISSEK, C. and ROTT, R. (1972). Inhibition of the multiplication of enveloped RNA viruses by glucosamine and 2-deoxy-D-glucose. J. Gen. Virol. 14, 251-259.

KAPLAN, A.S. and BEN-PORAT, T. (1964). Mode of replication of pseudorabies virus DNA. Virology 23, 90-95.

KAPLAN, A.S., ERICKSON, J.S. and BEN-PORAT, T. (1975). Synthesis of proteins in cells infected with herpesvirus. X. Proteins excreted by cells infected with herpes simplex virus types 1 and 2. Virology 64, 132-143.

KAPLAN, J., CALLEWAERT, D.M. and PETERSON, W.D. (1978). Expression of human T lymphocyte antigens by killer cells. J. Immunol. 121, 1,366-1,369.

KAPOOR, A.K., NASH, A.A., WILDY, P., PHELAN, J., MCLEAN, C.S. and FIELD, H.J. (1982). Pathogenesis of herpes simplex virus in congenitally athymic mice: the relative roles of cell-mediated and humoral immunity. J. Gen. Virol. 60, 225-233.

KARP, G.C. and SOLURSCH, M. (1974). Acid mucopolysaccharide metabolism, the cell surface and primary mesenchyme cell activity in the

sea urchin embryo. Dev. Biol. 41, 110-123.

KAZATCHKINE, M.D., FEARON, D.T., SILBERT, J.E. and AUSTEN, K.F. (1979). Surface-associated heparin inhibits zymosan-induced activation of the human alternative complement pathway by augmenting the regulatory action of the control proteins on particle-bound C3b. J. Exp. Med. 150, 1,202-1,215.

KEIR, H.M. (1968). Virus-induced enzymes in mammalian cells infected with DNA-viruses. In: "The Molecular Biology of Viruses". Eds. L.V. Crawford and M.G.P. Stoker. Cambridge University Press, Cambridge.

KEIR, H.M. and GOLD, E. (1963). Deoxyribonucleic acid nucleotidyltransferase and deoxyribonuclease from cultured cells infected with herpes simplex virus. Biochimica et Biophysica Acta. 72, 263-276.

KEIR, H.M., HAY, J., MORRISON, J.M. and SUBAK-SHARPE, J.H. (1966b). Altered properties of deoxyribonucleic acid nucleotidyltransferase after infection of mammalian cells with herpes simplex virus. nature 210, 369-371.

KEIR, H.M., SUBAK-SHARPE, J.H, SHEDDEN, W.I.H., WATSON, D.H. and WILDY, P. (1966a). Immunological evidence for a specific DNA polymerase produced after infection by herpes simplex virus. Virology 30, 154-157.

KELLER, R., PEITCHEL, R., GOLDMAN, J.M. and GOLDMAN, M. (1976). An IgG-Fc receptor induced in cytomegalovirus-infected human fibroblasts. J. Immunol. 116, 772-777.

KERR, C.L. and PENNINGTON, T.H. (1984). The effect of monensin on virion production and protein secretion in pseudorabies virus-infected cells. J. Gen. Virol. 65, 1,033-1,041.

KESSLER, M.J., MISE, T., GHAI, R.D. and BAHL, O.P. (1979). Structure and location of the O-glycosidic carbohydrate units of human chorionic gonadotropin. J. Biol. Chem. 254, 7,909-7,914.

KESSLER, S. (1975). Rapid isolatin of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction

sea urchin embryo. Dev. Biol. 41, 110-123.

KAZATCHKINE, M.D., FEARON, D.T., SILBERT, J.E. and AUSTEN, K.F. (1979). Surface-associated heparin inhibits zymosan-induced activation of the human alternative complement pathway by augmenting the regulatory action of the control proteins on particle-bound C3b. J. Exp. Med. 150, 1,202-1,215.

KEIR, H.M. (1968). Virus-induced enzymes in mammalian cells infected with DNA-viruses. In: "The Molecular Biology of Viruses". Eds. L.V. Crawford and M.G.P. Stoker. Cambridge University Press, Cambridge.

KEIR, H.M. and GOLD, E. (1963). Deoxyribonucleic acid nucleotidyltransferase and deoxyribonuclease from cultured cells infected with herpes simplex virus. Biochimica et Biophysica Acta. 72, 263-276.

KEIR, H.M., HAY, J., MORRISON, J.M. and SUBAK-SHARPE, J.H. (1966b). Altered properties of deoxyribonucleic acid nucleotidyltransferase after infection of mammalian cells with herpes simplex virus. nature 210, 369-371.

KEIR, H.M., SUBAK-SHARPE, J.H, SHEDDEN, W.I.H., WATSON, D.H. and WILDY, P. (1966a). Immunological evidence for a specific DNA polymerase produced after infection by herpes simplex virus. Virology 30, 154-157.

KELLER, R., PEITCHEL, R., GOLDMAN, J.M. and GOLDMAN, M. (1976). An IgG-Fc receptor induced in cytomegalovirus-infected human fibroblasts. J. Immunol. 116, 772-777.

KERR, C.L. and PENNINGTON, T.H. (1984). The effect of monensin on virion production and protein secretion in pseudorabies virus-infected cells. J. Gen. Virol. 65, 1,033-1,041.

KESSLER, M.J., MISE, T., GHAI, R.D. and BAHL, O.P. (1979). Structure and location of the O-glycosidic carbohydrate units of human chorionic gonadotropin. J. Biol. Chem. 254, 7,909-7,914.

KESSLER, S. (1975). Rapid isolatin of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction

of antibody-antigen complexes with protein A. J. Immunol. 115, 1,617-1,624.

KIEFF, E.D., BACHENHEIMER, S.L. and ROIZMAN, B. (1971). Size, composition and structure of the deoxyribonucleic acid of herpes simplex virus subtypes 1 and 2. J. Virol. 8, 125-132.

KIEFF, E.D., HOYER, B., BACHENHEIMER, S.L. and ROIZMAN, B. (1972). Genetic relatedness of type 1 and type 2 herpes simplex viruses. J. Virol. 9, 738-745.

KILBOURNE, E.D. (1959). Inhibition of influenza virus multiplication with a glucose antimetabolite (2-deoxy-D-glucose). Nature 183, 271-272.

KILLINGTON, R.A., YEO, J., HONNES, R.W., WATSON, D.H., DUNCAN, B.E., HALLIBURTON, I.W. and MUMFORD, J. (1977). Comparative analysis of the proteins and antigens of five herpesviruses. J. Gen. Virol. 37, 297-310.

KIT, S. and DUBBS, D.R. (1963). Acquisition of thymidine kinase activity by herpes simplex infected mouse fibroblast cells. Biochem. and Biophys. Res. Commun. 11, 55-59.

KLEMPERER, H.G., HAYNES, G.R., SHEDDON, W.I.H. and WATSON, D.H. (1967). A virus-specific thymidine kinase in BHK21 cells infected with herpes simplex virus. Virology 31, 120-128.

KLENK, H.D., ROTT, R., ORLICH, M. and BLODORN, J. (1975). Activation of influenza A viruses by trypsin treatment. Virology 68, 426-429.

KLENK, H.D., SCHOLTISSEK, C. and ROTT, R. (1972). Inhibition of glycoprotein biosynthesis of influenza virus by D-glucosamine and 2-deoxy-D-glucose. Virology 49, 723-734.

KNOPF, K.-W. (1979). Properties of herpes simplex virus DNA polymerase and characterisation of its associated exonuclease activity. Eur. J. Biochem. 98, 231-244.

KNOWLES, R.W. and PERSON, S. (1976). Effect of 2-deoxyglucose,

JOHNSON, D.C. and SPEAR, P.G. (1984). Evidence for translational regulation of herpes simplex virus type 1 gD expression. J. Virol. 51, 389-394.

JOHNSON, D.C., WITTELS, M. and SPEAR, P.G. (1984). Binding to cells of virosomes containing herpes simplex virus type 1 glycoproteins and evidence for fusion. J. Virol. 52, 238-247.

JONES, P.C. and ROIZMAN, B. (1979). Regulation of herpes virus macromolecular synthesis. VIII. The transcription programme consists of three phases during which both the extent and accumulation of RNA in the cytoplasm are regulated. J. Virol. 31, 299-314.

KALUZA, G., SCHMIDT, M.F.G. and SCHOLTISSEK, C. (1973). Effect of 2-deoxy-D-glucose on the multiplication of Semliki Forest Virus and the reversal of the block by mannose. Virology 54, 179-189.

KALUZA, G., SCHOLTISSEK, C. and ROTT, R. (1972). Inhibition of the multiplication of enveloped RNA viruses by glucosamine and 2-deoxy-D-glucose. J. Gen. Virol. 14, 251-259.

KAPLAN, A.S. and BEN-PORAT, T. (1964). Mode of replication of pseudorabies virus DNA. Virology 23, 90-95.

KAPLAN, A.S., ERICKSON, J.S. and BEN-PORAT, T. (1975). Synthesis of proteins in cells infected with herpesvirus. X. Proteins excreted by cells infected with herpes simplex virus types 1 and 2. Virology 64, 132-143.

KAPLAN, J., CALLEWAERT, D.M. and PETERSON, W.D. (1978). Expression of human T lymphocyte antigens by killer cells. J. Immunol. 121, 1,366-1,369.

KAPOOR, A.K., NASH, A.A., WILDY, P., PHELAN, J., MCLEAN, C.S. and FIELD, H.J. (1982). Pathogenesis of herpes simplex virus in congenitally athymic mice: the relative roles of cell-mediated and humoral immunity. J. Gen. Virol. 60, 225-233.

KARP, G.C. and SOLURSCH, M. (1974). Acid mucopolysaccharide metabolism, the cell surface and primary mesenchyme cell activity in the

sea urchin embryo. Dev. Biol. 41, 110-123.

KAZATCHKINE, M.D., FEARON, D.T., SILBERT, J.E. and AUSTEN, K.F. (1979). Surface-associated heparin inhibits zymosan-induced activation of the human alternative complement pathway by augmenting the regulatory action of the control proteins on particle-bound C3b. J. Exp. Med. 150, 1,202-1,215.

KEIR, H.M. (1968). Virus-induced enzymes in mammalian cells infected with DNA-viruses. In: "The Molecular Biology of Viruses". Eds. L.V. Crawford and M.G.P. Stoker. Cambridge University Press, Cambridge.

KEIR, H.M. and GOLD, E. (1963). Deoxyribonucleic acid nucleotidyltransferase and deoxyribonuclease from cultured cells infected with herpes simplex virus. Biochimica et Biophysica Acta. 72, 263-276.

KEIR, H.M., HAY, J., MORRISON, J.M. and SUBAK-SHARPE, J.H. (1966b). Altered properties of deoxyribonucleic acid nucleotidyltransferase after infection of mammalian cells with herpes simplex virus. nature 210, 369-371.

KEIR, H.M., SUBAK-SHARPE, J.H, SHEDDEN, W.I.H., WATSON, D.H. and WILDY, P. (1966a). Immunological evidence for a specific DNA polymerase produced after infection by herpes simplex virus. Virology 30, 154-157.

KELLER, R., PEITCHEL, R., GOLDMAN, J.M. and GOLDMAN, M. (1976). An IgG-Fc receptor induced in cytomegalovirus-infected human fibroblasts. J. Immunol. 116, 772-777.

KERR, C.L. and PENNINGTON, T.H. (1984). The effect of monensin on virion production and protein secretion in pseudorabies virus-infected cells. J. Gen. Virol. 65, 1,033-1,041.

KESSLER, M.J., MISE, T., GHAI, R.D. and BAHL, O.P. (1979). Structure and location of the O-glycosidic carbohydrate units of human chorionic gonadotropin. J. Biol. Chem. 254, 7,909-7,914.

KESSLER, S. (1975). Rapid isolatin of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction

of antibody-antigen complexes with protein A. J. Immunol. 115, 1,617-1,624.

KIEFF, E.D., BACHENHEIMER, S.L. and ROIZMAN, B. (1971). Size, composition and structure of the deoxyribonucleic acid of herpes simplex virus subtypes 1 and 2. J. Virol. 8, 125-132.

KIEFF, E.D., HOYER, B., BACHENHEIMER, S.L. and ROIZMAN, B. (1972). Genetic relatedness of type 1 and type 2 herpes simplex viruses. J. Virol. 9, 738-745.

KILBOURNE, E.D. (1959). Inhibition of influenza virus multiplication with a glucose antimetabolite (2-deoxy-D-glucose). Nature 183, 271-272.

KILLINGTON, R.A., YEO, J., HONNES, R.W., WATSON, D.H., DUNCAN, B.E., HALLIBURTON, I.W. and MUMFORD, J. (1977). Comparative analysis of the proteins and antigens of five herpesviruses. J. Gen. Virol. 37, 297-310.

KIT, S. and DUBBS, D.R. (1963). Acquisition of thymidine kinase activity by herpes simplex infected mouse fibroblast cells. Biochem. and Biophys. Res. Commun. 11, 55-59.

KLEMPERER, H.G., HAYNES, G.R., SHEDDON, W.I.H. and WATSON, D.H. (1967). A virus-specific thymidine kinase in BHK21 cells infected with herpes simplex virus. Virology 31, 120-128.

KLENK, H.D., ROTT, R., ORLICH, M. and BLODORN, J. (1975). Activation of influenza A viruses by trypsin treatment. Virology 68, 426-429.

KLENK, H.D., SCHOLTISSEK, C. and ROTT, R. (1972). Inhibition of glycoprotein biosynthesis of influenza virus by D-glucosamine and 2-deoxy-D-glucose. Virology 49, 723-734.

KNOPF, K.-W. (1979). Properties of herpes simplex virus DNA polymerase and characterisation of its associated exonuclease activity. Eur. J. Biochem. 98, 231-244.

KNOWLES, R.W. and PERSON, S. (1976). Effect of 2-deoxyglucose,

glucosamine and mannose on cell fusion and the glycoproteins of herpes simplex virus. J. Virol. 18, 644-651.

KO, G.K.W. and RAGHUPATHY, E. (1972). Glycoprotein biosynthesis in the developing rat brain. II. Microsomal galactosaminyltransferase utilizing endogenous and exogenous protein acceptors. Biochim. Biophys. Acta. 264, 129-143.

KOIDE, N. and MURAMATSU, T. (1974). Endo-beta-N-acetylglucosaminidase acting on carbohydrate moieties of glycoproteins. Purification and properties of the enzyme from Diplococcus pneumoniae. J. Biol. Chem. 249, 4,897-4,904.

KORNFELD, R. and KORNFELD, S. (1976). Comparative aspects of glycoprotein structure. Ann. Rev. Biochem. 45, 217-237.

KORNFELD, R. and KORNFELD, S. (1980). In: "The Biochemistry of Glycoproteins and Proteoglycans", pp.1-34. Ed. W. Lennarz.

KORNFELD, S., LI, E. and TABAS, I. (1978). The synthesis of complex-type oligosaccharides. II. Characterisation of the processing intermediates in the synthesis of the complex oligosaccharide units of the vesicular stomatitis virus G protein. J. Biol. Chem. 253, 7,771-7,778.

KOUSOULAS, K.G., BZIK, D.J., DELUCA, N. and PERSON, S. (1983). The effect of ammonium chloride and tunicamycin on the glycoprotein content and infectivity of herpes simplex virus type 1. Virology 125, 468-474.

KOUSOULAS, K.G., PELLET, P.E., PEREIRA, L. and ROIZMAN, B. (1984). Mutations affecting conformation or sequence of neutralizing epitopes identified by reactivity of viable plaques segregated from syn and ts domains of HSV-1 (F) gB gene. Virology 135, 379-394.

KOZAK, M. and ROIZMAN, B. (1974). Regulation of herpesvirus macromolecular synthesis: nuclear retention of non-translated viral RNA sequences. PNAS 71, 4,322-4,326.

KREIL, G. (1981). Transfer of proteins across membranes. Ann. Rev.

Biochem. 317-348.

KUDLER, L. and HYMAN, R.W. (1979). Exonuclease III digestion of herpes simplex virus DNA. Virology 92, 68-81.

LA BADIE, J.H., CHAPMAN, K.P. and ARONSON, N.N. Jr. (1975). Glycoprotein catabolism in rat liver. Lysosomal digestion of iodinated asialofetuin. Biochem. J. 154, 271-279.

LAMPORT, D.T.A. (1969). The isolation and partial characterisation of hydroxyproline-rich glycopeptides obtained by enzymic degradation of primary cell walls. Biochemistry 8, 1,155-1,163.

LANGELIER, Y., DECHAMPS, M. and BUTTIN, G. (1978). Analysis of dCMP deaminase and CDP reductase levels in hamster cells infected with herpes simplex virus. J. Virol. 26, 547-553.

LASKY, L.A., DOWBENKO, D., SIMONSEN, C.C. and BERMAN, P.W. (1984). Protection of mice from lethal herpes simplex virus infection by vaccination with a secreted form of cloned glycoprotein D. Biotechnology 2, 527-532.

LA THANGUE, N.B., SHRIVER, K., DAWSON, C. and CHAN, W.L. (1984). Herpes simplex virus infection causes the accumulation of a heat-shock protein. The EMBO Journal 3, 267-277.

LAZAROWITZ, S.G., GOLDBERG, A.R. and CHOPPIN, P.W. (1973). Proteolytic cleavage by plasmin of the HA polypeptides of influenza virus: host cell activation of serum plasminogen. Virology 56, 172-180.

LAZAROWITZ, S.G. and CHOPPIN, P.W. (1975). Enhancement of the infectivity of influenza A and B viruses by proteolytic cleavage of the haemagglutinin polypeptide. Virology 68, 440-454.

LEARY, K. and FRANCKE, B. (1984). The interaction of a topoisomerase-like enzyme from herpes simplex virus type 1-infected cells with non-viral circular DNA. J. Gen. Virol. 65, 1,341-1,350.

LEAVITT, R., SCHLESINGER, S. and KORNFELD, S. (1977). Tunicamycin inhibits glycosylation and multiplication of Sindbis and vesicular stomatitis

virus. J. Virol. 21, 375-385.

LEE, C.K. and KNIPE, D.M. (1983). Thermolabile in vivo DNA-binding activity associated with a protein encoded by mutants of herpes simplex virus type 1. J. Virol. 46, 909-919.

LEE, G.T.-Y., PARA, M.F. and SPEAR, P.G. (1982b). Location of the structural genes for glycoproteins gD and gE and for other polypeptides in the S component of herpes simplex virus type 1 DNA. J. Virol. 43, 41-49.

LEE, G.T.-Y., POGUE-GEILE, K.L., PEREIRA, L. and SPEAR, P.G. (1982a). Expression of herpes simplex virus glycoprotein C from a DNA fragment inserted into the thymidine kinase gene of this virus. PNAS 79, 6,612-6,616.

LEE, R.W.H. and HUTTNER, W.B. (1983). Tyrosine-O-sulphated proteins of PC12 pheochromocytoma cells and their sulfation by a tyrosylprotein sulfotransferase. J. Biol. Chem. 258, 11,326-11,334.

LEE, R.W.H. and HUTTNER, W.B. (1984). Tyrosylprotein sulfotransferase, a novel golgi enzyme involved in the tyrosine sulfation of proteins. J. Cell Biol. 99, 231a.

LEGLER, G. (1977). Glucosidases. Meth. Enzymology 46, 368-381.

LEHNER, T., WILTON, J.M.A. and SHILLITOE, E.J. (1975). Immunological basis for latency, recurrences, and putative oncogenicity of herpes simplex virus. Lancet II, 60-62.

LEINBACH, S.S., RENO,J.M., LEE, L.F., ISBELL, A.F. and BOEZI, J.A. (1976). Mechanism of phosphonoacetate inhibition of herpesvirus-induced DNA polymerase. Biochemistry 15, 426-430.

LEMANSKY, P., GIESELMAN, V., HASILIK, A. and VON FIGURA, K. (1984). Cathepsin D and beta-hexosaminidase synthesised in the presence of 1-deoxynojirimycin accumulate in the endoplasmic reticulum. J. Biol. Chem. 259, 10,129-10,135.

LI, E., TABAS, I. and KORNFELD, S. (1978). The synthesis of complex-type oligosaccharides. I. The structure of the lipid-linked oligosaccharide

precursor of the complex-type oligosaccharides of the vesicular stomatitis virus G protein. J. Biol. Chem. 253, 7,762-7,770.

LI, Y.T. and LI, S.-C. (1968). Studies on the glycosidases in jack bean meal. II. Separation of various glycosidases by isoelectric focussing. J. Biol. Chem. 243, 3,994-3,998.

LINGAPPA, V.R., LINGAPPA, J.R. and BLOBEL, G. (1979). Chicken ovalbumin contains an internal signal sequence. Nature 281, 117-121.

LITTLE, S.P., JOFRE, J.T., COURTNEY, R.J. and SCHAFFER, P.A. (1981). A virion-associated glycoprotein essential for infectivity of herpes simplex virus type 1. Virology 115, 149-160.

LITTLE, S.P. and SCHAFFER, P.A. (1981). Expression of the syncitial (syn) phenotype in HSV-1 strain KOS: genetic and phenotypic studies of mutants in two syn loci. Virology 112, 686-702.

LITTLER, E., PURIFOY, D.J.M., MINSON, A. and POWELL, K.L. (1983). Herpes simplex virus non-structural proteins. III. Function of the major DNA-binding protein. J. Gen. Virol. 64, 983-995.

LIU, L.F. (1983). DNA topoisomerases: enzymes that catalyze the breaking and rejoining of DNA. CRC Reviews in Biochem. 15, 1-24.

LIU, T., STETSON, B., TURCO, S.J., HUBBARD, C. and ROBBINS, P.W. (1979). Arrangement of glucose residues in the lipid-linked oligosaccharide precursor of asparaginyl oligosaccharides. J. Biol. Chem. 254, 4,554-4,559.

LOCKER, H. and FRENKEL, N. (1979). BamI, KpnI and SalI restriction enzyme maps of the DNAs of herpes simplex virus strains Justin and F: occurrence of heterogeneities in defined regions of the viral DNA. J. Virol. 32, 429-441.

LOFGREN, K.W., STEVENS, J., MARSDEN, H.S. and SUBAK-SHARPE, J.H. (1977). Temperature sensitive mutants of herpes simplex virus differ in the capacity to establish latent infection in mice. Virology 76, 440-443.

LONG, D., MADARA, T.J., PONCE DE LEON, M., COHEN, G.H., MONTGOMERY, P.C. and EISENBERG, R.J. (1984). Glycoprotein D

MACFARLANE, R.G. (1964). An enzyme cascade in the blood clotting mechanism and its function as a biochemical amplifier. Nature. 202, 498-499.

protects mice against lethal challenge with herpes simplex virus types 1 and 2. Infect. Immun. 37, 761-764.

MCDONALD, D. (1980). Herpes simplex virus-induced early proteins. Ph.D. Thesis, University of Glasgow.

MCGEOCH, D.J. (1984). Nature of animal virus genetic material. In: "The Microbe, Part 1: Virus". Eds. B.W.J. Mahy and J.R. Pattison. pp.75-107. Cambridge Univ. Press.

MCGEOCH, D.J. (1985). On the predictive recognition of signal peptide sequences. Virus Res. 3, 271-286.

MCGEOCH, D.J., DOLAN, A., DONALD, S. and RIXON, F.J. (1985). Sequence determination and genetic content of the short unique region in the genome of herpes simplex virus type 1. J. Mol. Biol. 181, 1-13.

MCGUIRE, E.J. and ROSEMAN, S. (1967). Enzymatic synthesis of the protein-hexosamine linkage in sheep submaxillary mucin. J. Biol. Chem. 242, 3,745-3,747.

MACKEM, S. and ROIZMAN, B. (1982a). Differentiation between alpha-promoter and regulator regions of herpes simplex virus 1: the functional domains and sequence of a movable regulator. PNAS 79, 4,917-4,921.

MACKEM, S. and ROIZMAN, B. (1982b). Structural features of the herpes simplex virus alpha gene 4, 0 and 27 promoter-regulatory sequences which confer alpha regulation on chimeric thymidine kinase genes. J. Virol. 44, 939-949.

MCKNIGHT, S.L. (1980). The nucleotide sequence and transcript map of the herpes simplex virus thymidine kinase gene. Nucl. Acids Res. 8, 5,949-5,964.

MCLAUCHLAN, J. and CLEMENTS, J.B. (1982). A 3' co-terminus of two early herpes simplex virus type 1 mRNAs. Nucl. Acids Res. 10, 501-512.

MCLAUCHLAN, J. and CLEMENTS, J.B. (1983). Organisation of the herpes simplex virus type 1 transcription unit encoding two early proteins with molecular weights of 140,000 and 40,000. J. Gen. Virol. 64, 997-

1,006.

MCLAUCHLAN, J., GAFFNEY, D., WHITTON, J.L. and CLEMENTS, J.B. (1985). The consensus sequence YGTGTTYY located downstream from the AATAAA signal is required for efficient formation of mRNA 3' termini. Nucl. Acids Res. 13, 1,347-1,368.

MCLEAN, C., BUCKMASTER, A., HANCOCK, D., BUCHAN, A., FULLER, A. and MINSON, A. (1982). Monoclonal antibodies to three non-glycosylated antigens of herpes simplex virus type 2. J. Gen. Virol. 63, 297-305.

MACPHERSON, I. and STOKER, M. (1962). Polyoma transformation of hamster cell clones - an investigation of genetic factors affecting cell competence. Virology 16, 147-151.

MCTAGGART, S.P., BURNS, W.H., WHITE, D.O. and JACKSON, D.C. (1978). Fc receptors induced by herpes simplex virus. J. Immunol. 121, 726-730.

MANSERVIGI, R., SPEAR, P.G. and BUCHAN, A. (1977). Cell fusion induced by herpes simplex virus is promoted and suppressed by different viral glycoproteins. PNAS 74, 3,913-3,917.

MAO, J.C.-H., ROBISHAW, E.E. and OVERBY, L.R. (1975). Inhibition of DNA polymerase from herpes simplex virus-infected Wi-38 cells by phosphonoacetic acid. J. Virol. 15, 1,281-1,283.

MARCIANO-CABRAL, R., DIX, R.D., CABRAL, G.A. and COURTNEY, R.J. (1977). Effects of cytochalasin B on the maturation of herpes simplex virus type 1: an ultrastructural investigation. Virology 76, 860-865.

MARINI, J.C., MILLER, K.G. and ENGLUND, P.T. (1980). Decatenation of kinetoplast DNA by topoisomerases. J. Biol. Chem. 255, 4,976-4,979.

MARLIN, S.D., HOLLAND, T.C., LEVINE, M. and GLORIOSO, J.C. (1985). Epitopes of herpes simplex virus type 1 glycoprotein gC are clustered in two distinct antigenic sites. J. Virol. 53, 128-136.

MARSDEN, H.S. (1980). Herpes simplex virus in latent infections.

Nature 288, 212-213.

MARSDEN, H.S., BUCKMASTER, A., PALFREYMAN, J.W., HOPE, R.G. and MINSON, A.C. (1984). Characterization of the 92,000 dalton glycoprotein induced by herpes simplex virus type 2. J. Virol. 50, 547-554.

MARSDEN, H.S., CROMBIE, I.K. and SUBAK-SHARPE, J.H. (1976). Control of protein synthesis in herpesvirus-infected cells: analysis of the polypeptides induced by wild-type and sixteen temperature-sensitive mutants of HSV strain 17. J. Gen. Virol. 31, 347-372.

MARSDEN, H.S., HAARR, L. and PRESTON, C.M. (1983). Processing of herpes simplex virus proteins and evidence that translation of thymidine kinase mRNA is initiated at three separate AUG codons. J. Virol. 46, 434-445.

MARSDEN, H.S., LANG, J., DAVISON, A.J., HOPE, R.G. and MACDONALD, D.M. (1982). Genomic location and lack of phosphorylation of the HSV immediate-early polypeptide IE12. J. Gen. Virol. 62, 17-27.

MARSDEN, H.S., STOW, N.D., PRESTON, V.G., TIMBURY, M.C. and WILKIE, N.M. (1978). Physical mapping of herpes simplex virus-induced polypeptides. J. Virol. 28, 624-642.

MARSHALL, R.D. (1972). Glycoproteins. Ann. Rev. Biochem. 42, 673-702.

MARSHALL, R.D. (1974). The nature and metabolism of the carbohydrate-peptide linkage of glycoproteins. Biochem. Soc. Symp. 40, 17-26.

MARSHALL, R.D. and NEUBERGER, A. (1964). Carbohydrates in protein. VIII. The isolation of 2-acetamido-1 (L-beta-aspartamido)-1,2-dideoxy-beta-D glucose from hen's egg albumin. Biochemistry 3, 1,596-1,600.

MARTIN, W.B., HAY, D., CRAWFORD, L.V., LEBOUVIER, G.L. and CRAWFORD, E.M. (1966). Characteristics of bovine mammillitis virus. J. Gen. Microbiol. 45, 325-332.

MATHEWS, M.B. (1975). Polyanionic proteoglycans. In: "Connective

tissue. Macromolecular structure and evolution". pp.93-125. Published by Springer-Verlag, Berlin, Heidelberg and New York.

MATTHEWS, R.E.F. (1982). Classification and nomenclature of viruses. Intervirology 17, 47-51.

MEAGER, A., UNGKITCHANUKIT, A. and HUGHES, R.C. (1976). Variants of hamster fibroblasts resistant to risin communis toxin (ricin). Biochem. J. 154, 113-124.

MEAGER, A., UNGKITCHANUKIT, A., NAIRN, R. and HUGHES, R.C. (1975). Ricin resistance in baby hamster kidney cells. Nature 257, 137-139.

MEYER, D.I., KRAUSE, E. and DOBBERSTEIN, B. (1982). Secretory protein translocation across membranes - the role of the 'docking protein'. Nature 297, 647-650.

MIYAMOTO, K. and MORGAN, C. (1971). Structure and development of viruses as observed in the electron microscope. IX. Entry and uncoating of herpes simplex virus. J. Virol. 8, 910-918.

MOCARSKI, E.S., POST, L.E. and ROIZMAN, B. (1980). Molecular engineering of the herpes simplex virus genome: insertion of a second L-S junction into the genome causes additional inversions. Cell 22, 243-255.

MOCARSKI, E.S. and ROIZMAN, B. (1981). Site-specific inversion sequence of the herpes simplex virus genome: domain and structural features. PNAS 78, 7,047-7,051.

MOCARSKI, E.S. and ROIZMAN, B. (1982a). Herpesvirus-dependent amplification and inversion of cell-associated viral thymidine kinase gene flanked by viral a sequences and linked to an origin of viral DNA replication. PNAS 79, 5,626-5,630.

MOCARSKI, E.S. and ROIZMAN, B. (1982b). Structure and role of the herpes simplex virus DNA termini in inversion, circularization and generation of virion DNA. Cell 31, 89-97.

MORGAN, C., ROSE, H.M., HOLDEN, M. and JONES, E.P. (1959).

Electron microscopic observations on the development of herpes simplex virus. J. Exp. Med. 110, 643-656.

MORGAN, C., ROSE, H.M. and MENDIS, B. (1968). Electron microscopy of herpes simplex virus. I. Entry. J. Virol. 2, 507-516.

MORRISON, J.M. and KEIR, H.M. (1968). A new DNA-exonuclease in cells infected with herpes simplex virus: partial purification and properties of the enzyme. J. Gen. Virol. 3, 337-347.

MORRISON, T.G. and LODISH, H.F. (1975). Site of synthesis of membrane and non-membrane proteins of vesicular stomatitis virus. J. Biol. Chem. 250, 6,955-6,962.

MORRISON, T.G. and SIMPSON, D. (1980). Synthesis, stability and cleavage of Newcastle Disease Virus glycoproteins in the absence of glycosylation. J. Virol. 36, 171-180.

MORSE, L., PEREIRA, L., ROIZMAN, B. and SCHAFFER, P.A. (1978). Anatomy of herpes simplex virus (HSV) DNA. X. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 x HSV-2 recombinants. J. Virol. 26, 389-410.

MORSE, L.S., BUCHMAN, T.G., ROIZMAN, B. and SCHAFFER, P.A. (1977). Anatomy of herpes simplex virus DNA. IX. Apparent exclusion of some parental DNA arrangements in the generation of intertypic (HSV-1 x HSV-2) recombinants. J. Virol. 24, 231-248.

MOSS, B., GERSHOWITZ, A., STRINGER, J.R., HOLLAND, L.E. and WAGNER, E.K. (1977). 5'-terminal and internal methylated nucleosides in herpes simplex virus type 1 mRNA. J. Virol. 23, 234-239.

MOSS, H., CHARTRAND, P., TIMBURY, M.C. and HAY, J. (1979). Mutant of herpes simplex virus type 2 with temperature-sensitive lesions affecting virion thermostability and DNase activity: identification of the lethal mutation and physical mapping of the nuc- lesion. J. Virol. 32, 140-146.

MULLER, M.T., BOLLES, C.S. and PARRIS, D.S. (1985). Association of

type 1 DNA topoisomerase with herpes simplex virus. J. Gen. Virol. 66, 1,565-1,574.

MUNK, K. and SAUER, G. (1964). Relationship between cell DNA metabolism and nucleocytoplasmic alterations in herpesvirus infected cells. Virology 22, 153-154.

MURAMATSU, T. (1971). Demonstration of an endo-glycosidase acting on a glycoprotein. J. Biol. Chem. 246, 5,535-5,537.

MURCHIE, M.-J. (1982). Sequence analysis of immediate-early gene regions in the S component of herpes simplex virus type 1 DNA. Ph.D. Thesis, University of Glasgow.

MURPHY, F.A., HARRISON, A.K. and WHITFIELD, S.G. (1967). Intranuclear formation of filaments in herpes virus hominis infection of mice. Arch. ges. Virusforsch. 21, 463-468.

NAKAMURA, K. and COMPANS, R.W. (1977). The cellular site of sulfation of influenza virus glycoproteins. Virology 79, 381-392.

NAKAMURA, K. and COMPANS, R.W. (1978). Effects of glucosamine, 2 deoxy-D-glucose and tunicamycin on glycosylation, sulphation and assembly of influenza viral proteins. Virology 84, 303-319.

NAKAMURA, K. and COMPANS, R.W. (1979). Host cell and virus strain dependent differences on oligosaccharides of haemagglutinin glycoproteins of influenza A viruses. Virology 95, 8-23.

NAKAMURA, Y., COSTA, J., TRALKA, T.S., YEE, C.L. and RABSON, A.S. (1978). Properties of the cell surface Fc-receptor induced by herpes simplex virus. J. Immunol. 121, 1,128-1,131.

NAZERIAN, K. (1974). DNA configuration in the core of marek's disease virus. J. Virol. 13, 1,148-1,150.

NEWTON, A.A. and STOKER, M.G.P. (1958). Change in nucleic acid content of HeLa cells infected with herpes simplex virus. Virology 5, 549-560.

NII, S. (1971a). Electron microscope observations on FL cells infected

with herpes simplex virus. I. Viral forms. Biken J. 14, 177-190.

NII, S. (1971b). Electron microscope observations on FL cells infected with herpes simplex virus. II. Envelopment. Biken J. 14, 325-348.

NII, S. and KAMAHORA, J. (1961). Cytopathic changes induced by herpes simplex virus. Bikens J., 4, 51-57.

NII, S., MORGAN, C. and ROSE, H.M. (1968a). Electron microscopy of herpes simplex virus. II. Sequence of development. J. Virol. 2, 517-536.

NII, S., MORGAN, C., ROSE, H.M. and HSU, K.C. (1968c). Electron microscopy of herpes simplex virus. IV. Studies with ferritin-conjugated antibodies. J. Virol. 2, 1,172-1,184.

NII, S., ROSENKRANZ, H.S., MORGAN, C. and ROSE, H.M. (1968b). Electron microscopy of herpes simplex virus. III. Effect of hydroxyurea. J. Virol. 2, 1,163-1,171.

NIWA, T., INOUE, S., TSURUOKA, T., KOAZE, Y. and NIIDA, T. (1970). "Nojirimycin" as a potent inhibitor of glucosidase. Agric. Biol. Chem. 34, 966-968.

NISHIOKA, Y. and SILVERSTEIN, S. (1978a). Alterations in the protein synthetic apparatus of Fried erythroleukemia cells infected with vesicular stomatitis virus or herpes simplex virus. J. Virol. 25, 422-426.

NISHIOKA, Y. and SILVERSTEIN, S. (1978b). Requirement of protein synthesis for the degradation of host mRNA in Friend erythroleukemia cells infected with herpes simplex virus type 1. J. Virol. 27, 619-627.

NOBLE, A.G., LEE, G.T.-Y., SPRAGUE, R., PARISH, M.L. and SPEAR, P.G. (1983). Anti-gD monoclonal antibodies inhibit cell fusion induced by herpes simplex virus type 1. Virology 129, 218-224.

NORRILD, B. (1980). Immunochemistry of herpes simplex virus glycoproteins. Current Topics in Microbial. Immunol. 90, 67-106.

NORRILD, B., BJERRUM, O.J., LUDWIG, H. and VESTERGAARD, B.F. (1978b). Analysis of herpes simplex virus type 1 antigens exposed on the surface of infected tissue culture cells. Virology 87, 307-316.

NORRILD, B., LUDWIG, H. and ROTT, R. (1978a). Identification of a common antigen of herpes simplex virus, bovine herpes mammillitis virus and B virus. J. Virol. 26, 712-717.

NORRILD, B. and PEDERSON, B. (1982). Effect of tunicamycin on the synthesis of herpes simplex virus type 1 glycoproteins and their expression on the cell surface. J. Virol. 43, 395-402.

NORRILD, B., SHORE, S.L. and NAHMIAS, A.J. (1979). Herpes simplex virus glycoproteins: participation of individual herpes simplex virus type 1 glycoprotein antigens in immunocytolysis and their correlation with previously identified glycopolypeptides. J. Virol. 32, 741-748.

NORRILD, B. and VESTERGAARD, B.F. (1979). Immunoelectrophoretic identification and purification of herpes simplex virus antigens released from infected cells in tissue culture. Intervirology 11, 104-110.

NORTH, G. (1985). Eukaryotic topoisomerases come into the limelight. Nature 316, 394-395.

NOSE, M. and WIGZELL, H. (1983). Biological significance of carbohydrate chains on monoclonal antibodies. PNAS 80, 6,632-6,636.

NOTARIANNI, E.L. and PRESTON, C.M. (1982). Activation of cellular stress protein genes by herpes simplex virus temperature-sensitive mutants which over-produce immediate early polypeptides. Virology 123, 113-122.

NOWELL, P.C. (1960). Phytohemagglutinin: an inhibitor of mitosis in culture of normal human leucocytes. Cancer Res. 20, 462-466.

OAKES, J.E. and LAUSCH, R.N. (1984). Monoclonal antibodies suppress replication of herpes simplex virus type 1 in trigeminal ganglia. J. Virol. 51, 656-661.

ODA, H., MORI, R., MIYAZONO, J. and IWASAKA, T. (1979). Effects of antimetabolites on the production of tubular structures in Vero cells infected with herpes simplex virus type 2. Arch. Virol. 62, 175-187.

O'FARRELL, P.Z., GOODMAN, H.M. and O'FARRELL, P.H. (1977). High resolution two-dimensional electrophoresis of basic as well as acidic

proteins. Cell 12, 1,133-1,141.

OGATA, M. and SHIGETA, S. (1979). Appearance of immunoglobulin G Fc receptors on cultured human cells infected with varicella-zoster virus. Infect. Immun. 26, 770-774.

O'HARE, P. and HAYWARD, G.S. (1985). Evidence for a direct role for both 175,000 and 110,000 molecular weight immediate-early proteins of herpes simplex virus in the transactivation of delayed-early promoters. J. Virol. 53, 751-760.

OLDEN, K., BERNARD, B.A., HUMPHRIES, M.J., YEO, T.-K., YEO, K.-T., WHITE, S.L., NEWTON, S.A., BAUER, H.C. and PARENT, J.B. (1985). Functions of glycoprotein glycans. Trends in Biological Sci. 10, 78-82.

OLOFSSON, S., BLOMBERG, J. and LYCKE, E. (1981b). O-glycosidic carbohydrate-peptide linkages of herpes simplex virus glycoproteins. Arch. Virol. 70, 321-329.

OLOFSSON, S., JEANSSON, S. and LYCKE, E. (1981a) Unusual lectin-binding properties of a herpes simplex virus type 1-specific glycoprotein. J. Virol. 38, 564-570.

OLOFSSON, S., LUNDSTROM, M., MARSDEN, H., JEANSSON, S. and VAHLNE, A. Characterization of a herpes simplex virus type 2-specified glycoprotein with affinity for N-acetylgalactosamine-specific lectins and its identification as g92K or gG. In Press. J. Gen. Virol.

OLSHEVSKY, U. and BECKER, Y. (1972). Surface glycopeptides in the envelope of herpes simplex virions. Virology 50, 277-279.

OMARY, M.B. and TROWBRIDGE, I.S. (1981). Covalent binding of fatty acids to the transferrin receptor in cultured human cells. J. Biol. Chem. 256, 4,715-4,718.

OPHEIM, D.J. and TOUSTER, O. (1978). Lysosomal alpha-D mannosidase of rat liver. Purification and comparison with the golgi and cytosolic alpha-D-mannosidases. J. Biol. Chem. 253, 1,017-1,023.

PADGETT, R.A., MOORE, D.F. and KINGSBURY, D.T. (1978) Herpes

simplex virus nucleic acid synthesis following infection of non-permissive XC cells. J. Gen. Virol. 40, 605-614.

PALFREYMAN, J.W., HAARR, L., CROSS, A., HOPE, R.G. and MARSDEN, H.S. (1983). Processing of herpes simplex virus type 1 glycoproteins: two dimensional gel analysis using monoclonal antibodies. J. Gen. Virol. 64, 873-886.

PAOLETTI, E., LIPINSKAS, B.R., SAMSONOFF, C., MERCER, S. and PANICALI, D. (1984). Construction of live vaccines using genetically engineered poxviruses: biological activity of vaccinia virus recombinants expressing the hepatitis virus surface antigen and the herpes simplex virus glycoprotein D. PNAS 81, 193-197.

PARA, M.F., BAUCKE, R.B. and SPEAR, P.G. (1980). Immunoglobulin G (Fc)-binding receptors on virions of herpes simplex virus type-1 and transfer of these receptors to the cell surface by infection. J. Virol. 34, 512-520.

PARA, M.F., BAUCKE, R.B. and SPEAR, P.G. (1982a). Glycoprotein gE of herpes simplex virus type 1: effect of anti-gE on virion infectivity and on virus-induced Fc-binding properties. J. Virol. 41, 129-136.

PARA, M.F., GOLDSTEIN, L. and SPEAR, P.G. (1982b). Similarities and differences in the Fc-binding glycoprotein (gE) of herpes simplex virus types 1 and 2 and tentative mapping of the viral gene for the glycoprotein. J. Virol. 41, 137-144.

PARA, M.F., ZEZULAK, K.M., CONLEY, A.J., WEINBERGER, M., SNITZER, K. and SPEAR, P.G. (1983). Use of monoclonal antibodies against two 75,000-molecular weight glycoproteins specified by herpes simplex virus type-2 in glycoprotein identification and gene mapping. J. Virol. 45, 1,223-1,227.

PARSONS, T.F. and PIERCE, J.G. (1980). Oligosaccharide moieties of glycoprotein hormones: bovine lutropin resists enzymatic deglycosylation because of terminal 0-sulfated N-acetylhexosamines. PNAS 77, 7,089-7,093.

PAULSON, J.C., PRIEELS, J.-P., GLASGOW, L.R. and HILL, R.L. (1978). Sialyl- and fucosyl-transferases in the biosynthesis of asparaginyl-linked oligosaccharides in glycoproteins. Mutually exclusive glycosylation by beta galactoside alpha 2-6 sialyltransferase and N acetylglucosaminide alpha 1-3 fucosyltransferase. J. Biol. Chem. 253. 5.617-5,624.

PAULSON, J.C., REARICK, J.I. and HILL, R.L. (1977). Enzymatic properties of beta-D-galactoside alpha 2-6 sialyltransferase from bovine colostrum. J. Biol. Chem. 252, 2,356-2,371.

PEAKE, M.L., NYSTROM, P. and PIZER, L.I. (1982). Herpesvirus glycoprotein synthesis and insertion into plasma membranes. J. Virol. 42, 678-690.

PELLET, P.E., KOUSOULAS, K.G., PEREIRA, L. and ROIZMAN, B. (1985). Anatomy of the herpes simplex virus 1 strain F glycoprotein B gene: primary sequence and predicted protein structure of the wild type and of monoclonal antibody-resistant mutants. J. Virol. 53, 243-253.

PENNINGTON, T.H., CARNAGIE, G.E. and ALCOCK, S.R. (1982). Specific secretion of polypeptides from cells infected with myxoma virus. J. Gen. Virol. 61, 115-120.

PENNINGTON, T.H. and MCCRAE, M.A. (1977). Processing of a pseudorabies virus-induced protein which is glycosylated, sulphated and excreted. J. Gen. Virol. 34, 155-165.

PERDUE, M.L., KEMP, M.C., RANDALL, C.C. and O'CALLAGHAN, D.J. (1974). Studies on the molecular anatomy of the L-M strain of equine herpes virus type 1. Proteins of the nucleocapsid and intact virion. Virology 59, 201-216.

PEREIRA, L., DONDERO, D., NORRILD, B. and ROIZMAN, B. (1981). Differential reactivity and processing of glycoproteins gA and gB of herpes simplex virus types 1 and 2 made in Vero and HEp-2 cells. PNAS 78, 5,202-5,206.

PEREIRA, L., KLASSEN, T. and BARINGER, J.R. (1980). Type-common

and type specific monoclonal antibody to herpes simplex virus type 1. J. Virol. 29, 724-732.

PEREIRA, L., WOLFF, M.H., FENWICK, M. and ROIZMAN, B. (1977). Regulation of herpesvirus macromolecular synthesis. V. Properties of polypeptides made in HSV-1 and HSV-2 infected cells. Virology 77, 733-749.

PERLMAN, D. and HALVORSON, H.O. (1983). A putative signal peptidase recognition site and sequence in eukaryotic and prokaryotic signal peptides. J. Mol. Biol. 167, 391-409.

PERSON, S., KOUSOULAS, K.G., KNOWLES, R.W., READ, G.S., HOLLAND, T.C., KELLER, P.M. and WARNER, S.C. (1982). Glycoprotein processing in mutants of HSV-1 that induce cell fusion. Virology 117, 293-306.

PESONEN, M. and KAARIAINEN, L. (1982). Incomplete complex oligosaccharides in Semliki Forest Virus envelope proteins arrested within the cell in the presence of monensin. J. Mol. Biol. 158, 213-230.

PIERCE, J.G. and PARSONS, T.F. (1981). Glycoprotein hormones: structure and function. Ann. Rev. Biochem. 50, 465-495.

PIGNATTI, P.F. and CASSAI, E. (1980). Analysis of herpes simplex virus nucleoprotein complexes extracted from infected cells. J. Virol. 36, 816-828.

PINTER, A. and COMPANS, R.W. (1975). Sulfated components of enveloped viruses. J. Virol. 16, 859-866.

PIZER, L.I., COHEN, G.H. and EISENBERG, R.J. (1980). Effect of tunicamycin on herpes simplex virus glycoproteins and infectious virus production. J. Virol. 34, 142-153.

PLESS, D.D. and LENNARZ, W.J. (1977). Enzymatic conversion of proteins to glycoproteins - evidence that Asn-X-Ser (Thr) is necessary for accepting oligosaccharides. PNAS 74, 134-138.

PLUMMER, G. and WATERSON, A.P. (1963). Equine herpesviruses. Virology 19, 412-416.

PLUMMER, T.H. and HIRS, C.W.H. (1964). On the structure of bovine pancreatic ribonuclease B. Isolation of a glycopeptide. J. Biol. Chem. 239, 2,530-2,538.

PODRAZKY, V., STEVEN, F.S., GRANT, M.E. and JACKSON, D.S. (1970). Protein-polysaccharide complexes of human intervertebral disc: chromatographic and isoelectric-focussing subfractionation. Biochimica et Biophysica Acta. 221, 549-558.

POGUE-GEILE, K.L., LEE, G.T.-Y., SHAPIRA, S.K. and SPEAR, P.G. (1984). Fine mapping of mutations in the fusion-inducing MP strain of herpes simplex virus type 1. Virology 136, 100-109.

POLLACK, L. and ATKINSON, P.H. (1983). Correlation of glycosylation forms with position in amino acid sequence. J. Cell Biol. 97, 293-300.

PONCE DE LEON, M., EISENBERG, R.J. and COHEN, G.H. (1977). Ribonucleotide reductase from herpes simplex virus (types 1 and 2) infected and uninfected KB cells: properties of the partially purified enzymes. J. Gen. Virol. 36, 163-173.

PONTECORVO, G. (1975). Production of mammalian somatic cell hybrids by means of polyethylene glycol treatment. Somatic Cell Genet. 1, 397-400.

POST, L.E., MACKEM, S. and ROIZMAN, B. (1981). Regulation of alpha genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with alpha gene promoters. Cell 24, 555-566.

POWELL, K.L., BUCHAN, A., SIM, C. and WATSON, D.H. (1974). Type-specific protein in herpes simplex virus envelope reacts with neutralizing antibody. Nature 249, 360-361.

POWELL, K.L. and COURTNEY, R.J. (1975). Polypeptides synthesised in herpes simplex virus type 2 infected HEp-2 cells. Virology 66, 217-228.

POWELL, K.L., LITTLER, E. and PURIFOY, D.J.M. (1981). Non-structural proteins of herpes simplex virus. II. Major virus-specific DNA-binding protein. J. Virol. 39, 894-902.

POWELL, K.L. and PURIFOY, D.J.M. (1976). DNA-binding proteins of cells infected by herpes simplex virus type 1 and type 2. Intervirology 7, 225-239.

POWELL, K.L, and PURIFOY, D.J.M. (1977). Non-structural proteins of herpes simplex virus. I. Purification of the induced DNA polymerase. J. Virol. 24, 618-626.

PREHM, P., SCHEID, A. and CHOPPIN, P.W. (1979). The carbohydrate structure of the glycoproteins of the paramyxovirus SV5 grown in bovine kidney cells. J. biol. Chem. 254, 9,669-9,677.

PRESTON, C.M. (1979a). Control of herpes simplex virus type 1 mRNA synthesis in cells infected with wild-type virus or the temperature-sensitive mutant tsK. J. Virol. 29, 275-284.

PRESTON, C.M. (1979b). Abnormal properties of an immediate early polypeptides in cells infected with herpes simplex virus type 1 mutant tsK. J. Virol. 32, 357-369.

PRESTON, C.M. and CORDINGLEY, M.G. (1982). mRNA and DNA-directed synthesis of herpes simplex virus-coded-exonuclease in Xenopus laevis oocytes. J. Virol. 43, 386-394.

PRESTON, C.M., CORDINGLEY, M.G. and STOW, N.D. (1984a). Analysis of DNA sequences which regulate the transcription of a herpes simplex virus immediate early gene. J. Virol. 50, 708-716.

PRESTON, C.M. and MCGEOCH, D.J. (1981). Identification and mapping of two polypeptides encoded within the herpes simplex virus type 1 thymidine kinase gene sequences. J. Virol. 38, 593-605.

PRESTON, V.G., DAVISON, A.J., MARSDEN, H.S., TIMBURY, M.C., SUBAK-SHARPE, J.H. and WILKIE, N.M. (1978). Recombinants between herpes simplex virus types 1 and 2: analysis of genome structures and expression of immediate-early polypeptides. J. Virol. 28, 499-517.

PRESTON, V.G. and FISHER, F.B. (1984). Identification of the herpes simplex virus type 1 gene encoding the dUTPase. Virology 138, 58-68.

PRESTON, V.G., PALFREYMAN, J.W. and DUTIA, B.M. (1984b). Identification of a herpes simplex virus type 1 polypeptide which is a component of the virus-induced ribonucleotide reductase. J. Gen. Virol. 65, 1,457-1,466.

PRICE, R.W., WALZ, M.A., WOHLENBERG, C.R. and NOTKINS, A.L. (1975). Latent infection of sensory ganglia with herpes simplex virus: efficacy of immunization. Science 188, 938-940.

PRICER, W.E. Jr. and ASHWELL, G. (1976). Subcellular distribution of a mammalian hepatic binding protein specific for asialoglycoproteins. J. Biol. Chem. 251, 7,539-7,544.

PRIEELS, J.-P., BEYER, T.A. and HILL, R.L. (1977). Human milk fucosyltransferases. Biochem. Soc. Trans. 5, 838-839.

PROUDFOOT, N.J. and BROWNLEE, G.G. (1974). 3' non-coding region sequences in eukaryotic messenger RNA. Nature 263, 211-214.

PROUDFOOT, N.J. and BROWNLEE, G.G. (1976). Sequence at the 3' end of globin mRNA shows homology with immunoglobulin light chain mRNA. Nature 252, 359-362.

PUGA, A., ROSENTHAL, J.D., OPENSHAW, H. and NOTKINS, A.L. (1978). Herpes simplex virus DNA and mRNA sequences in acutely and chronically infected trigeminal ganglia of mice. Virology 89, 102-111.

PURIFOY, D.J.M. and BENYESH-MELNICK, M. (1974). DNA polymerase induction by DNA-negative temperature-sensitive (ts) mutants of herpes simplex virus type 2 (HSV-2). Abstracts Ann. Meet. Amer. Soc. Microbiol., p.234.

PURIFOY, D.J.M. and BENYESH-MELNICK, M. (1975). DNA polymerase induction by DNA-negative temperature-sensitive mutants of herpes simplex virus type 2. Virology 68, 374-386.

PURIFOY, D.J.M. and POWELL, K.L. (1976). DNA-binding proteins induced by herpes simplex virus type 2 in HEp-2 cells. J. Virol. 19, 717-731.

QUINLAN, M.P. and KNIPE, D.M. (1985). Stimulation of expression of a herpes simplex virus DNA-binding protein by two viral functions. Mol. Cell. Biol. 5, 957-963.

QUINN, J.P. (1984). Sequence of the DNA polymerase locus in the genome of herpes simplex virus type 1. Ph.D. Thesis. University of Glasgow.

QUINN, P., GRIFFITHS, G. and WARREN, G. (1983). Dissection of the golgi complex. II. Density separation of specific golgi functions in virally infected cells treated with monensin. J. Cell Biol. 96, 851-856.

RAFIELD, L.F. and KNIPE, D.M. (1984). Characterization of the major mRNAs transcribed from the genes for glycoprotein B and DNA-binding protein ICP8 of herpes simplex virus type 1. J. Virol. 49, 960-969.

RAHMAN, A.A., TESCHNER, M., SETHI, K.K. and BRANDIS, H. (1976). Appearance of IgG (Fc) receptor(s) on culture human fibroblasts infected with human cytomegalovirus. J. Immunol. 117, 253-258.

RANDALL, R.E. and HONESS, R.W. (1980). Proteins of herpesvirus saimiri: identification of two virus polypeptides released into the culture medium of productively infected cells. J. Gen. Virol. 51, 445-449.

RANDALL, R.E., KILLINGTON, R.A. and WATSON, D.H. (1980). Glycoproteins with type common and type specific antigenic sites excreted from cells infected with herpes simplex virus types 1 and 2. J. Gen. Virol. 48, 297-310.

RAO, A.K. and MENDICINO, J. (1978). Influence of glycopeptide structure on the regulation of galactosyltransferase activity. Biochem. 17, 5,632-5,638.

READING, C.L., PENHOET, E.E. and BALLOU, C.E. (1978). Carbohydrate structure of the vesicular stomatitis virus glycoprotein. J. Biol. Chem. 253, 5,600-5,612.

RECTOR, J.T., LAUSCH, R.N. and OAKES, J.E. (1984). Identification of infected cell-specific monoclonal antibodies and their role in host

RIXON,F.J.,ATKINSON,M.A.and HAY,J.(1983).Intranuclear distribution of herpes simplex virus type-2 DNA synthesis. Examination by light and electron microscopy. J.Gen.Virol.64,2087-2092.

resistance to ocular herpes simplex virus type 1 infection. J. Gen. Virol. 65, 657-661.

RIXON, F.J. (1977). Studies on herpes simplex virus DNA synthesis. Ph.D. Thesis. University of Glasgow.

RIXON, F.J. and CLEMENTS, J.B. (1982). Detailed structural analysis of two spliced immediate early mRNAs. Nucl. Acids Res. 10, 2,241-2,256.

RIXON, F.J. and MCGEOCH, D.J. (1984). A 3' co-terminal family of mRNAs from the herpes simplex virus type 1 short region: two overlapping reading frames encode unrelated polypeptides one of which has a highly reiterated amino acid sequence. Nucl. Acids Res. 12, 2,473-2,487

RIXON, F.J. and MCGEOCH, D.J. (1985). Detailed analysis of the mRNAs mapping in the short unique region of herpes simplex virus type 1. Nucl. Acids Res. 13, 953-973.

ROBERTS, P.L., DUNCAN, B.E., RAYBOULD, J.G. and WATSON, D.H. (1985). Purification of herpes simplex virus glycoproteins B and C using monoclonal antibodies and their ability to protect mice against lethal challenge. J. Gen. Virol. 66, 1,073-1,085.

ROITT, I. (1984). The immunoglobulins. In: "Essential Immunology", Chapter 2, pp.21-46. Published by Blackwell Scientific Publications.

ROITT, I. (1984). Interaction of antigen and antibody. In: "Essential Immunology", Chapter 6, pp.145-178. Published by Blackwell Scientific Publications.

ROIZMAN, B. (1962). Polykaryocytosis. Cold Spring Harbor Symp. Quant. Biol. 27, 327-342.

ROIZMAN, B. (1979). The structure and isomerization of herpes simplex virus genomes. Cell 16, 481-494.

ROIZMAN, B. (1985). The family herpesviridae: general description, taxonomy and classification. In: "The Herpesviruses", Volume 1, pp.1-23. Ed. B. Roizman. Plenum Press, New York.

ROIZMAN, B., BORGMAN, G.S. and KAMALI-ROUSTA, M. (1965).

Macromolecular synthesis in cells infected with herpes simplex virus. Nature 206, 1,374, 1,375.

ROIZMAN, B., CARMICHAEL, L.E., DEINHARDT, F., DE THE, G.B., NAHMIAS, A.J., PLOWRIGHT, W., RAPP, F., SHELDRICK, P., TAKAHASHI, M. and WOLFE, K. (1981). Herpes-viridae: Definition, provisional nomenclature and taxonomy. Intervirology 16, 201-217.

ROIZMAN, B., CARMICHAEL, S., DE THE, G.B., MASIC, M., NAHMIAS, A.J., PLOWRIGHT, W., RAPP, F., SHELDRICK, P., TAKAHASHI, M. TERNI, M. and WOLFE, K. (1978). Provisional classification of herpesviruses. In: "Oncogenesis of Herpesviruses", III, Part 2, pp.1,079-1,082. Eds. G. de The, F. Rapp and W. Henle. IARC Scientific Publications, No. 24, Lyon.

ROIZMAN, B. and FURLONG, D. (1974). The replication of herpesviruses. In: "Comprehensive Virology", Volume 3, pp.229-403. Eds. H. Fraenkel-Conrat and R.R. Wagner. Plenum Press, New York.

ROIZMAN, B., KOZAK, M., HONESS, R.W. and HAYWARD, G. (1974). Regulation of herpesvirus macromolecular synthesis: evidence for multi-level regulation of herpes simplex 1 RNA and protein synthesis. Cold Spring Harbor Symp. Quant. Biol. 39, 687-701.

ROIZMAN, B., NORRILD, B., CHAN, C. and PEREIRA, L. (1984). Identification and preliminary mapping with monoclonal antibodies of a herpes simplex virus 2 glycoprotein lacking a known type-1 counterpart. Virology 133, 242-247.

ROMERO, P., DATERNA, R. and SCHWARZ, R.T. (1983). N-methyl-1-deoxynojirimycin, a novel inhibitor of glycoprotein processing and its effect on Fowl Plague Virus maturation. Virology 130, 238-242.

ROSA, P., FUMAGALLI, G., ZANINI, A. and HUTTNER, W.B. (1985). The major tyrosine-sulphated protein of the bovine anterior pituitary is a secretory protein present in gonadotrophs, thyrotrophs, mammotrophs and corticotrophs. J. Cell Biol. 100, 928-937.

ROSE, J.K., ADAMS, G.A. and GALLIONE, C.J. (1984). The presence of cysteine in the cytoplasmic domain of the vesicular stomatitis virus glycoprotein is required for palmitate addition. PNAS 81, 2,050-2,054.

ROSEVEAR, J.W. and SMITH, E.L. (1961). Glycopeptides: I. Isolation and properties of glycopeptides from a fraction of human gamma-globulin. J. Biol. Chem. 236, 425-435.

ROSNER, M.R., GRINNA, L.S. and ROBBINS, P.W. (1980). Differences in glycosylation patterns of closely related murine leukemia viruses. PNAS 77, 67-71.

ROTHMAN, J.E. (1981). The golgi apparatus: two organelles in tandem. Science 213, 1,212-1,219.

ROTHMAN, J.E. (1985). The compartmental organization of the golgi apparatus. Scientific American 253, 84-95.

ROTHMAN, J.E. and LENARD, J. (1977). Membrane asymetry. The nature of membrane asymetry provides clues to the uzzle of how membranes are assembled. Science 195, 743-753.

ROTHMAN, J.E. and LODISH, H.F. (1977). Synchronized transmembrane insertion and glycosylation of a nascent membrane protein. Nature 269, 775-780.

ROTHMAN, J.E., MILLER, R.L. and URBANI, L.J. (1984b). Intercompartmental transport in the golgi complex is a dissociative process: facile transfer of membrane protein between two golgi populations. J. Cell Biol. 99, 260-271.

ROTHMAN, J.E., URBANI, L.J. and BRANDS, R. (1984a). Transport of protein between cytoplasmic membranes of fused cells: correspondence to processes reconstituted in a cell-free system. J. Cell Biol. 99, 248-259.

RUBENSTEIN, A.S., GRAVELL, M. and DARLINGTON, R. (1972). Protein kinase in enveloped herpes simplex virions. Virology 50, 287-290.

RUYECHAN, W.T. (1983). The major herpes simplex virus DNA-binding protein holds single-stranded DNA in an extended conformation. J. Virol.

46, 661-666.

RUYECHAN, W.T., MORSE, L.S., KNIPE, D.M. and ROIZMAN, B. (1979). Molecular genetics of herpes simplex virus. II. Mapping of the major viral glycoproteins and of the genetic loci specifying the social behaviour of infected cells. J. Virol. 29, 677-697.

SABATINI, D.D., KREIBICH, G., MORIMOTO, T. and ADESNIK, M. (1982). Mechanisms for the incorporation of proteins in membranes and organelles. J. Cell Biol. 92, 1-22.

SANDERS, P.G., WILKIE, N.M. and DAVISON, A.J. (1982). Thymidine kinase deletion mutants of herpes simplex virus type 1. J. Gen. Virol. 63, 277-295.

SANDRI-GOLDIN, R.M., LEVINE, M. and GLORIOSO, J.C. (1981). Method for induction of mutations in physically defined regions of the herpes simplex virus genome. J. Virol. 38, 41-49.

SARMIENTO, M., HAFFEY, M. and SPEAR, P.G. (1979). Membrane proteins specified by herpes simplex virus. III. Role of glycoprotein VP7 (B2) in virion infectivity. J. Virol. 29, 1,149-1,158.

SARMIENTO, M. and SPEAR, P.G. (1979). Membrane proteins specified by herpes simplex virus. IV. Conformation of the virion glycoprotein designated VP7 (B2). J. Virol. 29, 1,159-1,167.

SAUNIER, B., KILKER, R.D., TKACZ, J.S., QUARONI, A. and HERSKOVICS, A. (1982). Inhibition of N-linked complex oligosaccharide formation by 1-deoxynojirimycin, an inhibitor of processing glucosidases. J. Biol. Chem. 257, 14,155-14,161.

SCHACHTER, H. (1979). Glycoprotein biosynthesis. In: "The glycoconjugates", Volume II, pp.87-181. Eds. H.I. Horowitz and W. Pigman.

SCHACHTER, H. and ROSEMAN, S. (1980). In: "The biochemistry of glycoproteins and proteoglycans", pp.85-160. Ed. W. Lennarz. Plenum Press, New York.

SCHENKEL-BRUNNER, H., PROHASKA, R. and TUPPY, H. (1975).

Action of glycosyl transferases upon "Bombay" (Oh) erythrocytes. Conversion to cells showing blood-group H and A specificities. Eur. J. Biochem. 56, 591-594.

SCHLOMAI, J., FRIEDMAN, A. and BECKER, Y. (1976). Replicative intermediates of herpes simplex DNA. Virology 69, 647-659.

SCHMIDT, M.F.G., BRACHA, M. and SCHLESINGER, M.J. (1979). Evidence for covalent attachment of fatty acids to Sindbis virus glycoproteins. PNAS 76, 1,687-1,691.

SCHMIDT, M.F.G. and SCHLESINGER, M.J. (1979). Fatty acid binding to vesicular stomatitis virus glycoprotein: a new type of post-translational modification of the viral glycoprotein. Cell 17, 813-819.

SCHMIDT, M.F.G. and SCHLESINGER, M.J. (1980). Relation of fatty acid attachment to the translation and maturation of vesicular stomatitis virus and sindbis virus membrane glycoproteins. J. Biol. Chem. 255, 3,334-3,339.

SCHNEWEIS, K.E. (1962). Serologische untersuchengen zur typendifferenzierung des herpes-virus hominis. Z. Immunitaetsforsch. Exp. Ther. 124, 24-48.

SCHRIER, R.D., PIZER, L.I. and MOORHEAD, J.W. (1983). Type-specific delayed hypersensitivity and protective immunity induced by isolated herpes simplex virus glycoprotein. J. Immunol. 130, 1,413-1,418.

SCHUMAKER, V.N., CALCOTT, M.A., SPIEGELBERG, H.L. and MULLER-EBERHARD, H.J. (1976). Ultracentrifuge studies of the binding of IgG of different subclasses to the CIq subunit of the first component of complement. Biochemistry 15, 5,175-5,181.

SCHWARTZ, J. and ROIZMAN, B. (1969). Concerning the egress of herpes simplex virus from infected cells: electron and light microscopic observations. Virology 38, 42-49.

SCHWARZ, R.T., ROHRSCHNEIDER, J.M. and SCHMIDT, M.F.G. (1976). Suppression of glycoprotein formation of Semliki Forest, Influenza and Avian Sarcoma Virus by tunicamycin. J. Virol. 19, 782-791.

SCHWARZ, R.T., SCHMIDT, M.F. and DATEMA, R. (1979). Inhibition of glycosylation of viral glycoproteins. Biochem. Soc. Trans. 7, 322-326.

SEKIZAWA, T., OPENSHAW, H., WOHLENBERG, C. and NOTKINS, A.L. (1980). Latency of herpes simplex virus in absence of neutralizing antibody: model for reactivatin. Science 210, 1,026-1,028.

SERAFINI-CESSI, F. and CAMPADELLI-FIUME, G. (1981). Studies on benzhydrazone, a specific inhibitor of herpesvirus glycoprotein synthesis. Size distribution of glycopeptides and endo-beta-N-acetylglucosaminidase H treatment. Arch. Virol. 70, 331-343.

SERAFINI-CESSI, F., DALL'OLIO, F. and CAMPADELLI-FIUME, G. (1984a). O-glycosylation of herpes simplex virus glycoproteins carrying high mannose glycans. Biochem. Soc. Trans. 12, 328-329.

SERAFINI-CESSI, F., DALL'OLIO, F., PEREIRA, L. and CAMPADELLI-FIUME, G. (1984b). Processing of N-linked oligosaccharides from precursor- to mature-form herpes simplex virus type 1 glycoprotein gC. J. Virol. 51, 838-844.

SERAFINI-CESSI, F., DALL'OLIO, F., SCANNAVINI, M. and CAMPADELLI-FIUME, G. (1983a). Processing of herpes simplex virus-1 glycans in cells defective in glycosyl transferases of the golgi system: relationship to cell fusion and virion egress. Virology 131, 59-70.

SERAFINI-CESSI, F., DALL'OLIO, F., SCANNAVINI, M., COSTANZO, F. and CAMPADELLI-FIUME, G. (1983b). N-acetylgalactosaminyltransferase activity involved in O-glycosylation of herpes simplex virus type 1 glycoproteins. J. Virol. 48, 325-329.

SERAFINI-CESSI, F., MALAGOLINI, N., DALL'OLIO, F., PEREIRA, L. and CAMPADELLI-FIUME, G. (1985). Oligosaccharide chains of herpes simplex virus type 2 glycoprotein gG2. Arch. Biochem. Biophys. 240, 866-876.

SETHI, K.K., OMATA, Y. and SCHNEWEIS, E.E. (1983). Protection of mice from fatal herpes simplex virus type 1 infection by adoptive transfer of cloned virus specific and H2-restricted cytotoxic T lymphocytes. J.

Gen. Virol. 64, 443-447.

SHARON, N. and LIS, H. (1972). Lectins: cell-agglutinating and sugar-specific proteins. Science 177, 949-959.

SHARON, N. and LIS, H. (1982). Glycoproteins. In: "The Proteins", 3rd Edition, Volume 5, pp.1-144. Eds. H. Neurath and R.L. Hill. Academic Press, New York and London.

SHELDRICK, P. and BERTHELOT, N. (1974). Inverted repetitions in the chromosome of herpes simplex virus. Cold Spring Harbor Symp. Quant. Biol. 39, 667-678.

SHEN, L. GROLLMAN, E.F. and GINSBERG, V. (1967). An enzymatic basis for secretor status and blood group substance specificity in humans. PNAS 59, 224-230.

SHIDA, H. and DALES, S. (1981). Biogenesis of vaccinia: carbohydrate of the haemagglutinin molecule. Virology 111, 56-72.

SHOWALTER, S.D., ZWEIG, M. and HAMPAR, B. (1981). Monoclonal antibodies to herpes simplex virus type 1 proteins, including the immediate-early protein ICP4. Infect. Immun. 34, 684-692.

SILVERSTEIN, S., BACHENHEIMER, S.L., FRENKEL, N. and ROIZMAN, B. (1973). Relationship between post-transcriptional adenylation of herpes simplex RNA and messenger RNA abundance. PNAS 70, 2,101-2,104.

SILVERSTEIN, S., MILLETTE, R., JONES, P. and ROIZMAN, B. (1976). RNA synthesis in cells infected with herpes simplex virus. XII. Sequence complexity and properties of RNA differing in extent of adenylation. J. Virol. 18, 977-991.

SKARE, J. and SUMMERS, W.C. (1977). Structure and function of herpesvirus genomes. II. EcoRI, XbaI and HindIII endonuclease cleavage sites on herpes simplex virus type 1 DNA. Virology 76, 581-595.

SMILEY, J.R., FONG, B.S. and LEUNG, W.-C. (1981). Construction of a double-jointed herpes simplex viral DNA molecule: inverted repeats are required for segment inversion, and direct repeats promote deletions.

Virology 113, 345-362.

SMITH, J.D. and DE HARVEN, E. (1973). Herpes simplex virus and cytomegalovirus replication in WI-38 cells. I. Sequence of virus replication. J. Virol. 12, 919-930.

SMITH, K.O. (1964). Relationship between the envelope and the infectivity of herpes simplex virus. Proc. Soc. Exp. Biol. Med. 115, 814-816.

SMITH, M.G. (1954). Propagation of salivary gland virus of the mouse in tissue culture. Proc. Soc. Exp. Biol. Med. 86. 435-440.

SMITH, M.G. (1956). Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease. Proc. Soc. Exp. Biol. Med. 92, 424-430.

SPAETE, R.R. and FRENKEL, N. (1982). The herpes simplex virus amplicon: a new eukaryotic defective virus cloning-amplifying vector. Cell 30, 295-304.

SPEAKE, B.K. and WHITE, D.A. (1979). The effect of tunicamycin on the glycosylation of lactating-rabbit mammary glycoproteins. Biochem. J. 180, 481-489.

SPEAR, P.G. (1976). Membrane proteins specified by herpes simplex viruses. I. Identification of four glycoprotein precursors and their products in type-1-infected cells. J. Virol. 17, 991-1,008.

SPEAR, P.G. and ROIZMAN, B. (1972). Proteins specified by herpes simplex virus. V. Purification and structural proteins of the herpesvirion. J. Virol. 9, 143-159.

SPIRO, R.G. (1962). Studies on the monosaccharide sequence of the serum glycoprotein fetuin. J. Biol. Chem. 237, 646-652.

SPIRO, R.G. (1970). Glycoproteins. Annual Review of Biochemistry, 599-638.

SPIRO, R.G. (1973). Glycoproteins. Adv. Protein Chem. 27, 349-467.

SPIRO, R.G., SPIRO, M.J. and BHOYROO, V.D. (1979). Processing of

carbohydrate units of glycoproteins. Characterisation of a thyroid glucosidase. J. Biol. Chem. 254, 7,659-7,667.

SPRING, S.B. and ROIZMAN, B. (1968). Herpes simplex virus products in productive and abortive infections. III. Differentiation of infectious virus derived from nucleus and cytoplasm with respect to stability and size. J. Virol. 2, 979-985.

STALLCUP, K.C. and FIELDS, B.N. (1981). The replication of Measles Virus in the presence of tunicamycin. Virology 108, 391-404.

STEIN, S., TODD, P. and MAHONEY, J.H. (1970). Infectivity of herpes simplex virus with and without envelopes. Canadian J. Microbiol. 16, 953-957.

STEINER, D.F., QUINN, P.S., CHANN, S.J., MARSH, J. and TAGER, H.S. (1980). Processing mechanisms in the biosynthesis of protein. Ann. N.Y. Acad. Sci. 343, 1-16.

STENBERG, R.M. and PIZER, L.I. (1982). Herpes simplex virus induced changes in cellular and adenovirus RNA metabolism in an adenovirus type 5-transformed cell line. J. Virol. 42, 474-487.

STEVELY, W.S. (1975). Virus-induced proteins in pseudorabies-infected cells. II. Proteins of the virion and nucleocapsid. J. Virol. 16, 944-950.

STEVENS, J.G. (1975). Latent herpes simplex virus and the nervous system. Current Topics in Microbiol. and Immunol. 70, 31-50.

STEVENS, J.G. and COOK, M.L. (1971). Latent herpes simplex virus in spinal ganglia of mice. Science 173, 843-845.

STEVENS, J.G. and COOK, M.L. (1974). Maintenance of latent herpatic infection. An apparent role of anti-viral IgG. J. Immunol. 113, 1,685-1,693.

STOCKERT, R.J., MORELL, A.G. and SCHEINBERG, I.H. (1974). Mammalian hepatic lectin. Science 186, 365-366.

STOFFEL, W., BLOBEL, G. and WALTER, P. (1981). Synthesis in vitro and translocation of apolipoprotein A1 across microsomal vesicles. Eur. J.

Biochem. 120, 519-522.

STOHRER, R. and HUNTER, E. (1979). Inhibition of Rous Sarcoma Virus replication by 2-deoxyglucose and tunicamycin: identification of an unglycosylated env gene product. J. Virol. 32. 412-419.

STOKER, M.G.P. (1958). Mode of intercellular transfer of herpes virus. Nature 182, 1,525-1,526.

STOKER, M.G.P. and NEWTON, A.A. (1959). The effect of herpes virus on HeLa cells dividing parasynchronously. Virology 7, 438-448.

STOW, N.D. (1978). Physical mapping of herpes simplex virus type 1 mutations. Ph.D. Thesis. University of Glasgow.

STOW, N.D. (1982). Localisation of an origin of DNA replication within the TR_S/IR_S repeated region of the herpes simplex virus type 1 genome. The EMBO J. 1, 863-867.

STOW, N.D. and MCMONAGLE, E.C. (1983). Characterisation of the TR_S/IR_S origin of DNA replication of herpes simplex virus type 1. Virology 130, 427-438.

STOW, N.D., MCMONAGLE, E.C. and DAVISON, A.J. (1983). Fragments from both termini of the herpes simplex virus type 1 genome contain signals required for the encapsidation of viral DNA. Nucl. Acids Res. 11, 8,205-8,220.

STRAUSS, J.H., BURGE, B.W. and DARNELL, J.E. (1970). Carbohydrate content of the membrane protein of sindbis virus. J. Mol. Biol. 47, 437-448.

STROBEL-FIDLER, M. and FRANCKE, B. (1980). Alkaline deoxyribonuclease induced by herpes simplex virus type 1: composition and properties of the purified enzyme. Virology 103, 493-501.

STROUS, G.J.A.M. (1979). Initial glycosylation of proteins with acetylgalactosaminylserine linkages. PNAS 76, 2,694-2,698.

SUBAK-SHARPE, J.H., BURK, R.R., CRAWFORD, L.V., MORRISON, J.M., HAY, J. and KEIR, H.M. (1966). An approach to evolutionary

relationships of mammalian DNA viruses through analysis of the pattern of nearest neighbour base sequence. Cold Spring Harbor Symp. Quant. Biol. 31, 737-748.

SUGINO, W.M. and KINGSBURY, D.T. (1976). DNA homologies between strains of herpes simplex virus. Virology 71, 605-608.

SUNSTRUM, J., CHRISP, C., LEVINE, M. and GLORIOSO, J. (1985). Deletion of herpes simplex virus type 1 glycoprotein C alters neuropathogenicity. Tenth International Herpesvirus Workshop, Ann Arbor, USA, p.249.

SVEDA, M.M., MARKOFF, L.J. and LAI, C.-J. (1982). Cell surface expression of the influenza virus haemagglutinin requires the hydrophobic carboxy terminal sequences. Cell 30, 649-656.

SWAIN, M.A., PEET, R.W. and GALLOWAY, D.A. (1985). Characterization of the gene encoding herpes simplex virus type 2 glycoprotein C and comparison with the type 1 counterpart. J. Virol. 53, 561-569.

SWANSTROM, R.I. and WAGNER, E.K. (1974). Regulation of synthesis of herpes simplex virus type 1 mRNA during productive infection. Virology 60, 522-533.

SYDISKIS, R.J. and ROIZMAN, B. (1966). Polysomes and protein synthesis in cells infected with a DNA virus. Science 153, 76-78.

SYDISKIS, R.J. and ROIZMAN, B. (1967). The disaggregation of host polyribosomes in productive and abortive infection with herpes simplex virus. Virology 32, 678-686.

TABAS, I. and KORNFELD, S. (1978). The synthesis of complex type oligosaccharides. III. Purification of an alpha-D-mannosidase activity involved in a late stage of processing of complex-type oligosaccharides. J. Biol. Chem. 253, 7,779-7,786.

TABAS, I. and KORNFELD, S. (1979). Purification and characterizatiin of a rat liver golgi alpha-mannosidase capable of processing asparagine-linked

oligosaccharides. J. Biol. Chem. 254, 11,655-11,663.

TAI, T., YAMASHITA, K., ARAKAWA, M., KOIDE, N., MURAMATSU, T., IWASHITA, S., INOUE, Y. and KOBATA, A. (1975). Structural studies of two ovalbumin glycopeptides in relation to the endo-beta-N-acetylglucosaminidase specificity. J. Biol. Chem. 250, 8,569-8,575.

TAKATSUKI, A., KAWAMURA, K., OKINA, M., KODAMA, Y., ITO, T. and TAMURA, G. (1977). The structure of tunicamycin. Agric. Biol. Chem. 41, 2,307-2,309.

TAKATSUKI, A., KOHNO, K. and TAMURA, G. (1975). Inhibition of biosynthesis of polyisoprenol sugars in chick embryo microsomes by tunicamycin. Agr. and Biol. Chem. 39, 2,089-2,091.

TARENTINO, A.L. and MALEY, F. (1974). Purification and properties of an endo-beta-N-acetylglucosaminidase from Streptomyces griseus. J. Biol. Chem. 249, 811-817.

TARENTINO, A.L., PLUMMER, T.H. Jr. and MALEY, F. (1972). A re-evaluation of the oligosaccharide sequence associated with ovalbumin. J. Biol. Chem. 247, 2,629-2,631.

TARTAKOFF, A.M. and VASSALLI, P. (1977). Plasma membrane immunoglobulin secretion: arrest is accompanied by alterations in the golgi complex. J. Exp. Med. 146, 1,332-1,345.

TARTAKOFF, A.M. and VASSALLI, P. (1978). Comparative studies of intracellular transport of secretory proteins. J. Cell Biol. 79, 694-707.

TKACZ, J.S. and LAMPEN, J.O. (1975). Tunicamycin inhibition of polyisoprenyl N-acetylglucosaminyl pyrophosphate formation in calf-liver microsomes. Biochem. Biophys. Res. Commun. 65, 248-257.

TENSER, R.B. and DUNSTAN, M.E. (1979). Herpes simplex virus thymidine kinase expression in infection of the trigeminal ganglion. Virology 99, 417-422.

TENSER, R.B., MILLER, R.L. and RAPP, F. (1979). Trigeminal ganglion infection by thymidine kinase-negative mutants of herpes simplex virus.

Science 205, 915-917.

TERNI, M. and ROIZMAN, B. (1970). Variability of herpes simplex virus: isolation of two variants from simultaneous eruptions at different sites. J. Infect. Dis. 121, 212-216.

THOMAS, D.B. and WINZLER, R.J. (1971). Structure of glycoproteins of human erythrocytes. Alkali stable oligosaccharides. Biochem. J. 124, 55-59.

TIMBURY, M.C. (1971). Temperature sensitive mutants of herpes simplex virus type 2. J. Gen. Virol. 9, 143-159.

TIMBURY, M.C. and SUBAK-SHARPE, J.H. (1973). Genetic interactions between temperature-sensitive mutants of type 1 and 2 herpes simplex virus. J. Gen. Virol. 18, 347-357.

TIMBURY, M.C., THERIAULT, A. and ELTON, R.A. (1974). A stable syncytial mutant of herpes simplex type 2 virus. J. Gen. Virol. 23, 219-224.

TOGNON, M., FURLONG, D., CONLEY, A.J. and ROIZMAN, B. (1981). Molecular genetics of herpes simplex virus. V. Characterization of a mutant defective in ability to form plaques at low temperatures and in a viral function which prevents accumulation of coreless capsids at nuclear pores late in infection. J. Virol. 40, 870-880.

TRIMBLE, R.B., MALEY, F. and CHU, F.K. (1983). Glycoprotein biosynthesis in yeast. Protein conformation affects processing of high mannose oligosaccharides on carboxypeptidase Y and invertase. J. Biol. Chem. 258, 2,562-2,567.

TULLO, A.B., SHIMELD, C., EASTY, D.L. and DARVILLE, J.M. (1983). Distribution of latent herpes simplex virus infection in human trigeminal ganglion. Lancet, i, 353.

TULSIANI, D.R.P., HARRIS, T.M. and TOUSTER, O. (1982b). Swainsonine inhibits the biosynthesis of complex glycoproteins by inhibition of golgi mannosidase II. J. Biol. Chem. 257, 7,936-7,939.

TULSIANI, D.R.P., HUBBARD, S.C., ROBBINS, P.W. and TOUSTER, O. (1982a). Alpha-D-mannosidases of rat liver golgi membranes. Mannosidase II is the GlcNAc-Man_5-cleaving enzyme in glycoprotein biosynthesis and mannosidases IA and IB are the enzymes converting MAN_9 precursors to MAN_5 intermediates. J. Biol. Chem. 257, 3,660-3,668.

TULSIANI, D.R.P., OPHEIM, D.J. and TOUSTER, O. (1977). Purification and characterisation of alpha-D-mannosidase from rat liver golgi membranes. J. Biol. Chem. 252, 3,227-3,233.

UCHIDA, N., SMILOWITZ, M. and TANZER, M.L. (1979). Monovalent ionophores inhibit secretion of procollegen and fibronectin from cultured human fibroblasts. PNAS 76, 1,868-1,872.

VAHLNE, A., SVENNERHOLM, B. and LYCKE, E. (1979). Evidence for herpes simplex type-selective receptors on cellular plasma membranes. J. Gen. Virol. 44, 217-225.

VAHLNE, A., SVENNERHOLM, B., SANDBERG, M., HAMBERGER, A. and LYCKE, E. (1980). Differences in attachment between herpes simplex type 1 and type 2 viruses to neurons and glial cells. Infect. Immun. 28, 675-680.

VAUGHAN, P.J., PURIFOY, D.J.M. and POWELL, K.L. (1985). DNA-binding protein associated with herpes simplex virus DNA polymerase. J. Virol. 53, 501-508.

VERNON, S.K., LAWRENCE, W.C. and COHEN, G.H. (1974). Morphological components of herpesvirus. I. Intercapsomeric fibrils and the geometry of the capsid. Intervirology 4, 237-248.

VESTERGAARD, B.F., BJERRUM, O.J., NORRILD, B. and GRAUBALLE, P.C. (1977). Crossed immunoelectrophoretic studies of the solubility and immunogenicity of herpes simplex virus antigens. J. Virol. 24, 82-90.

WADSWORTH, S., HAYWARD, G.-S. and ROIZMAN, B. (1976). Anatomy of herpes simplex virus DNA. V. Terminally repetitive sequences. J. Virol. 17, 503-512.

WALSH,K.A.(1975).Unifying concepts among proteases. In Proteases and Biological Control. Ed. by Reich,E., Rifkin,D.B. and Shaw,E. pp.1-13. Published by Cold Spring Harbor Conferences on Cell Proliferation.

WADSWORTH, S., JACOB, R.J. and ROIZMAN, B. (1975). Anatomy of herpes simplex virus DNA. II. Size, composition and arrangement of inverted terminal repetitions. J. Virol. 15, 1,487-1,497.

WAECHTER, C.J. and LENNARZ, W.J. (1976). The role of polyprenol-linked sugars in glycoprotein synthesis. Ann. Rev. Biochem. 45, 95-112.

WAGNER, E.K. (1985). Individual HSV transcripts. Characterization of specific genes. In: "The Herpesviruses", III, pp.45-104.

WAGNER, E.K. and ROIZMAN, B. (1969a). Ribonucleic acid synthesis in cells infected with herpes simplex virus. I. Patterns of ribonucleic acid synthesis in productively infected cells. J. Virol. 4, 36-46.

WAGNER, E.K. and ROIZMAN, B. (1969b). RNA synthesis in cells infected with herpes simplex virus. II. Evidence that a class of viral mRNA is derived from a high molecular weight precursor synthesised in the nucleus. PNAS 64, 626-633.

WAGNER, E.K. and SUMMERS, W.C. (1978). Structure of the joint region and the termini of the DNA of herpes simplex virus type 1. J. Virol. 27, 374-387.

WAGNER, E.K., SWANSTROM, R.I. and STAFFORD, M.G. (1972). Transcription of the herpes simplex virus genome in human cells. J. Virol. 10, 675-682.

WAGNER, M.J., SHARP, J.A. and SUMMERS, W.C. (1981). Nucleotide sequence of the thymidine kinase gene of herpes simplex virus type 1. PNAS 78, 1,441-1,445.

WALTER, P. and BLOBEL, G. (1980). Purification of a membrane-associated protein complex required for translocation across the endoplasmic reticulum. PNAS 77, 7,112-7,116.

WALTER, P. and BLOBEL, G. (1981). Translocation of proteins across the endoplasmic reticulum. II. Signal recognition protein (SRP) mediates the selective binding to microsomal membranes of in vitro assembled polysomes synthesising secretory proteins. J. Cell Biol. 91, 551-556.

WALTER, P. and BLOBEL, G. (1982). Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. Nature 299, 691-698.

WALTER, P., JACKSON, R.C., MARCUS, M.M., LINGAPPA, V.R. and BLOBEL, B. (1979). Tryptic dissection and reconstitution of translocation activity for nascent presecretory proteins across microsomal membranes. PNAS 76, 1,795-1,799.

WARREN, K.G., DEVLIN, M., GILDEN, D.H., WROBLEŃSKA, Z., BROWN, S.M., SUBAK-SHARPE, J.H. and KOPROWSKI, H. (1977). Isolation of latent herpes simplex virus from human trigeminal ganglia including ganglia from one patient with multiple sclerosis. Lancet, ii, 637-639.

WATKINS, J.F. (1964). Adsorption of sensitised sheep erythrocytes to HeLa cells infected with herpes simplex virus. Nature 202, 1,364-1,365.

WATKINS, W.M. (1972). Blood group specific substances. In: "Glycoproteins". pp.830-891. Ed. A. Gottschalk. Elsevier, Amsterdam.

WATKINS, W.M. and MORGAN, W.T.J. (1952). Neutralization of the anti-H agglutinin in eel serum by simple sugars. Nature 169, 825-826.

WATSON, D.H. and WILDY, P. (1969). The preparation of 'monoprecipitin' antisera to herpes virus specific antigens. J. Gen. Virol. 4, 163-168.

WATSON, K., STEVENS, J.G., COOK, M.L. and SUBAK-SHARPE, J.H. (1980). Latency competence of thirteen HSV-1 temperature-sensitive mutants. J. Gen. Virol. 49, 149-159.

WATSON, R.J. (1983). DNA sequence of the herpes simplex virus type 2 glycoprotein D gene. Gene 26, 307-312.

WATSON, R.J. and CLEMENTS, J.B. (1978). Characterization of transcription-deficient temperature-sensitive mutants of herpes simplex virus type 1. Virology 91, 364-379.

WATSON, R.J. and CLEMENTS, J.B. (1980). A herpes simplex virus type 1 function continuously required for early and late virus RNA synthesis. Nature 285, 329-330.

WATSON,R.J.,PRESTON,C.M.and CLEMENTS,J.B.(1979).Separation and characterisation of herpes simplex virus type-1 immediate-early mRNAs. J.Virol. 31, 42-52.

normal human immunoglobulin. J. Immunol. 116, 1,566-1,570.

WESTMORELAND, D. and WATKINS, J.F. (1974). The IgG receptor induced by herpes simplex virus: studies using radioiodinated IgG. J. Gen. Virol. 24, 167-178.

WHITTON, J.L. and CLEMENTS, J.B. (1984). The junction between the repetitive and the short unique sequences of the herpes simplex virus genome are determined by the polypeptide-coding regions of two spliced immediate-early mRNAs. J. Gen. Virol. 65, 451-466.

WILCOX, K.W., KOHN, A., SKLYANSKAYA, E. and ROIZMAN, B. (1980). Herpes simplex virus phosphoproteins. I. Phosphate cycles on and off some viral polypeptides and can alter their affinity for DNA. J. Virol. 33, 167-182.

WILDY, P., FIELD, H.J. and NASH, A.A. (1982). Classical herpes latency revisited. In: "Symposium 33, Society for General Microbiology", pp.133-167. Eds. B.W.J. Mahy, A.C. Minson and G.K. Darby. Cambridge University Press, London.

WILDY, P., RUSSELL, W.C. and HORNE, R.W. (1960). The morphology of herpes simplex virus. Virology 12, 204-222.

WILDY, P., SMITH, C., NEWTON, A.A. and DENDY, P. (1961). Quantitative cytological studies on HeLa cells infected with herpes virus. Virology 15, 486-500.

WILKIE, N.M. (1973). The synthesis and substructure of herpes virus DNA: the distribution of alkali-labile single strand interruptions in HSV-1 DNA. J. Gen. Virol. 21, 453-467.

WILKIE, N.M. (1976). Physical maps for herpes simplex virus type 1 DNA for restriction endonucleases HindIII, Hpa-I and X-bad. J. Virol. 20, 222-233.

WILKIE, N.M. and CORTINI, R. (1976). Sequence arrangement in herpes simplex virus type 1 DNA: identification of terminal fragments in restriction endonuclease digests and evidence for inversion in redundant and

unique sequences. J. Virol. 20, 211-221.

WILKIE, N.M., DAVISON, A., CHARTRAND, P., STOW, N.D., PRESTON, V.G. and TIMBURY, M.C. (1979). Recombination in herpes simplex virus: mapping of mutations and analysis of intertypic recombinants. Cold Spring Harbor Symp. Quant. Biol. 43, 827-840.

WILKIE, N.M., STOW, N.D., MARSDEN, H.S., PRESTON, V., CORTINI, R., TIMBURY, M.C. and SUBAK-SHARPE, J.H. (1977). Physical mapping of herpes simplex virus coded functions and polypeptides by marker rescue and analysis of HSV-1/HSV-2 intertypic recombinants. In: "Oncogenesis and Herpesviruses", III, Volume 1, pp.11-31. Eds. G. deThe, W. Henle and F. Rapp. IARC Scientific Publications, No. 24, Lyon.

WILLIAMS, M.V. (1984). Demonstration of a herpes simplex virus type 2-induced deoxyuridine triphosphate nucleotidohydrolase in infected KB cells and in biochemically transformed HeLa cells. J. Gen. Virol. 65, 209-213.

WILSON, J.R., WILLIAMS, D. and SCHACHTER, H. (1976). The control of glycoprotein synthesis: N-acetylglucosamine linkage to a mannose residue as a signal for the attachment of L-fucose to the asparagine-linked N-acetylglucosamine residue of glycopeptide from alpha-acid glycoprotein. Biochem. Biophys. Res. Commun. 72, 909-916.

WOHLRAB, F. and FRANCKE, B. (1980). Deoxypyrimidine triphosphatase activity specific for cells infected with herpes simplex virus type 1. PNAS 77, 1,872-1,876.

WOHLRAB, F., GARRETT, B.K. and FRANCKE, B. (1982). Control of expression of the herpes simplex virus-specified deoxypyrimidine triphosphatase in cells infected with mutants of herpes simplex virus types 1 and 2 and intertypic recombinants. J. Virol. 43, 935-942.

WOLF, K. and DARLINGTON, R.W. (1971). Channel catfish virus: a new herpesvirus of ictalurid fish. J. Virol. 8, 525-533.

YASUDA, J. and MILGROM, F. (1968). Hemadsorption by herpes simplex infected cell cultures. Int. Arch. Allergy 33, 151-170.

YEWDELL, J.W. and GERHARD, W. (1981). Antigenic characterization of viruses by monoclonal antibodies. Ann. Rev. Microbiol. 35, 185-206.

YOST, C.S., HEDGPETH, J. and LINGAPPA, V.R. (1983). A stop transfer sequence confers predictable transmembrane orientation to a previously secreted protein in cell-free systems. Cell 34, 759-766.

ZEZULAK, K.M. and SPEAR, P.G. (1983). Characterization of a herpes simplex virus type 2 75,000 molecular weight glycoprotein antigenically related to herpes simplex virus type 1 glycoprotein C. J. Virol. 47, 553-562.

ZEZULAK, K.M. and SPEAR, P.G. (1984). Mapping of the structural gene for the herpes simplex virus type 2 counterpart of herpes simplex virus type 1 glycoprotein C and identification of a type 2 mutant which does not express this glycoprotein. J. Virol. 49, 741-747.

ZWEIG, M., HEILMAN, C.J. Jr. and HAMPAR, B. (1979). Identification of disulphide-linked protein complexes in the nucleocapsids of herpes simplex virus type 2. Virology 94, 442-450.

ZWEIG, M., HEILMAN, C.J. Jr., RABIN, H. and HAMPAR, B. (1980). Shared antigenic determinants between two distinct classes of proteins in cells infected with herpes simplex virus. J. Virol. 35, 644-652.

ZWEIG, M., SHOWALTER, S.D., BLADEN, S.V., HEILMAN, J.C. Jr. and HAMPAR, B. (1983). Herpes simplex virus type 2 glycoprotein gF and type 1 glycoprotein gC have related antigenic determinants. J. Virol. 47, 185-192.

ZWEIG, M., SHOWALTER, S.D., SIMMS, D.J. and HAMPAR, B. (1984). Antibodies to a synthetic oligopeptide that react with herpes simplex virus type 1 and 2 glycoprotein C. J. Virol. 51, 430-436.