However, the actual mechanism of dietary life-span extension is not understood, and only a few investigators have examined the relation between dietary manipulation and the functional manifestations of senescence (*6–8*). Since degeneration and reduced responsiveness of the striatal dopaminergic system is one of the best documented functional impairments of the aging mammalian brain (*9–13*), we have examined the effects of dietary restriction on the normal age-related loss of dopamine receptors from the rat corpus striatum.

In Fig. 1A the Scatchard analysis (*14*) of ^{3}H-labeled amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene binding to crude striatal membrane preparations shows that there is a progressive decrease in dopamine receptor concentration (abscissa intercept) but not affinity (dissociation constant, K_D, is equal to the negative reciprocal of the slope) as the rats age from 3 to 24 months. Table 1 indicates that this reduction is statistically significant ($P < .01$) and amounts to about 40 percent over the adult life-span.

When male rats are subjected to a restricted diet by being given access to food only every other day, the mean life-span of the Wistar strain used in these experiments is increased from 99 to 138 weeks (*15*). Figure 1B shows that the concentrations of striatal dopamine receptors in the brains of 24-month-old animals maintained on restricted diets from weaning are substantially higher than those of 24-month-old control rats given free access to food everyday. Table 1 contains data from a number of separate analyses of data from rats on restricted diets, and confirms that receptor concentrations in these animals are indeed significantly different from 24-month-old controls ($P < .001$) and essentially comparable to those of 3- to 6-month-old control rats. Binding affinities are equivalent in all groups.

It therefore appears that dietary restriction, in the form of alternate days of feeding and fasting, substantially retards the loss of striatal dopamine receptors that is responsible for altered dopaminergic control of physiological and behavioral functions in senescent rats (*9–12*). The preservation of receptor levels typical of young adults late into the life-span is in concert with the approximately 40 percent increase in mean survival time effected by the present dietary manipulation (*15*). Although prolonged retention of striatal dopamine receptors probably represents a consequence rather than a cause of dietarily increased rat life-span, such findings suggest exciting possibilities, especially if they are applicable to man.

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Differentiation of Respiratory and Abortigenic Isolates of Equine Herpesvirus 1 by Restriction Endonucleases

Abstract. *Viruses classified by immunologic criteria as equine herpesvirus 1 cause respiratory disease and abortion in horses. Restriction endonuclease analyses of the DNA's of viruses from animals with respiratory disease and from aborted fetuses show that the patterns for respiratory viruses, while similar to each other, are entirely different from the patterns for fetal viruses. It is therefore proposed that the DNA restriction endonuclease patterns of fetal and respiratory viruses analyzed in this study be designated as prototypic of equine herpesvirus 1 and 4, respectively.*

Three distinct herpesviruses designated as equine herpesviruses 1, 2, and 3 (EHV1, EHV2, and EHV3) have been isolated from horses (*1*). The viruses classified by their immunologic specificity as EHV1 are the major cause of acute viral upper respiratory disease and of abortion in horses. It has been stated from time to time that viruses isolated from respiratory infections (R) differ from viruses isolated from aborted fetuses (F) with respect to antigenicity, host range in vitro, plaque size, growth rate in vitro and in vivo, and epidemiology (*2–5*). A central question, therefore, was whether the R and F isolates could be differentiated with respect to nucleotide sequence of their DNA's, as reflected by the distribution of restriction endonuclease cleavage sites.

In addition, because of the serious economic losses caused by EHV1, particularly from epizootic abortion (abortion "storms"), several attempts have been made to control such losses by vaccination; one of the three EHV1 viruses licensed for vaccine use was associated with neurologic disease in 486 of 60,000 recipients of the vaccine and was subsequently withdrawn. We showed earlier that no two epidemiologically unrelated herpes simplex viruses are identical with respect to the number and distribution of the restriction endonuclease cleavage sites in their DNA's and that these patterns can be used as fingerprints for tracing the spread of herpes simplex viruses in the population (*6–8*). It was of interest, therefore, to determine whether the viruses classified as EHV1 varied, and whether the DNA fingerprinting technique could be used to differentiate between vaccine and wild-type viruses.

We now report that the R and F viruses are clearly different. Furthermore, although the number of isolates was small, no two epidemiologically unrelated viruses within each of the groups appeared to have identical DNA fragment patterns.

The origin and designation of the EHV1, EHV2, and EHV3 viruses are listed in Table 1. The viruses were grown in equine kidney cell cultures (passages 5 to 7), in equine dermal (EDerm) cell line (*9*), or in Vero cells. Viral DNA was extracted from the cytoplasm or from whole infected cells. The procedures for

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extraction, purification, and limit digestion of these DNA's with Eco RI, Bam HI, Kpn I, and Xba I restriction endonucleases were the same as those for analyses of herpes simplex virus DNA (*7, 8*). The patterns of electrophoretically separated DNA fragments in agarose gels show the following: (i) The electrophoretic mobilities of the DNA fragments obtained with Eco RI, Bam HI, and Kpn I (Fig. 1, A to C) and with Xba I (not shown) indicate that the F isolates (B, D, and 03), although differing somewhat from each other in one or more restriction endonuclease cleavage sites, are distinctly different from the R isolates (39, 56, and 57). (ii) The fragment patterns of the DNA's of the vaccine strains N and Q, known to be of fetal origin, resemble but are not identical to the B, D, and 03 fetal isolates. Vaccine strain 12, reported to have been isolated from a bovine fetus (*10*), can be readily identified as an equine fetal virus. Furthermore, the three vaccine viruses yielded unique patterns indicating that they can be differentiated on the basis of the presence or absence of specific restriction endonuclease cleavage sites. (iii) Among the respiratory viruses, 56 and 57 appear to be identical; this would be expected since the viruses were isolated from the same outbreak of respiratory infection among horses in the United States. Strain 39, although isolated from Australia, is readily grouped with respiratory viruses 56 and 57 on the basis of the comigration of many of its DNA fragments. (iv) Although the total number of viruses analyzed is small, the data suggest that the restriction endonuclease DNA patterns may be used to identify or trace the spread of particular viruses in the equine populations.

Table 1. Equine herpesviruses analyzed by restriction endonucleases.

Virus origin and designation	Place	Year	References
Equine respiratory tract			
39	Victoria, Australia	1967	(*14, 15*)
56	Iowa	1976	(*16*)
57	Iowa	1976	(*16*)
Equine fetus			
B	Kentucky	1950	(*15, 17*)
D	Kentucky	1950	(*9, 17*)
03	Kansas	1980	(*16*)
Q	California	1964	(*15, 18*)
N	Poland	1965	(*15, 19*)
Bovine fetus			
12	Illinois	1975	(*10*)
Equine respiratory tract			
EHV2 LK	England	1963	(*9, 20*)
Equine genital tract			
EHV3 1118	Kentucky	1968	(*9, 21*)

Our results indicate that the viruses currently classified as EHV1 represent two distinct groups. Whereas the differences within each group represent variability in the presence or absence of a limited number of restriction endonuclease cleavage sites, the differences between the two groups probably reflect total displacement of restriction endonuclease cleavage sites. The situation that we have encountered with the respiratory and fetal isolates appears to be similar to that encountered with herpes simplex viruses 1 and 2. Although the herpes simplex viruses are immunologically related and share at least 50 percent of their DNA sequences, with good matching of base pairs, they differ in the predominant site of isolation (*11*) and in the location of restriction endonuclease cleavage sites on their DNA's (*12*). On

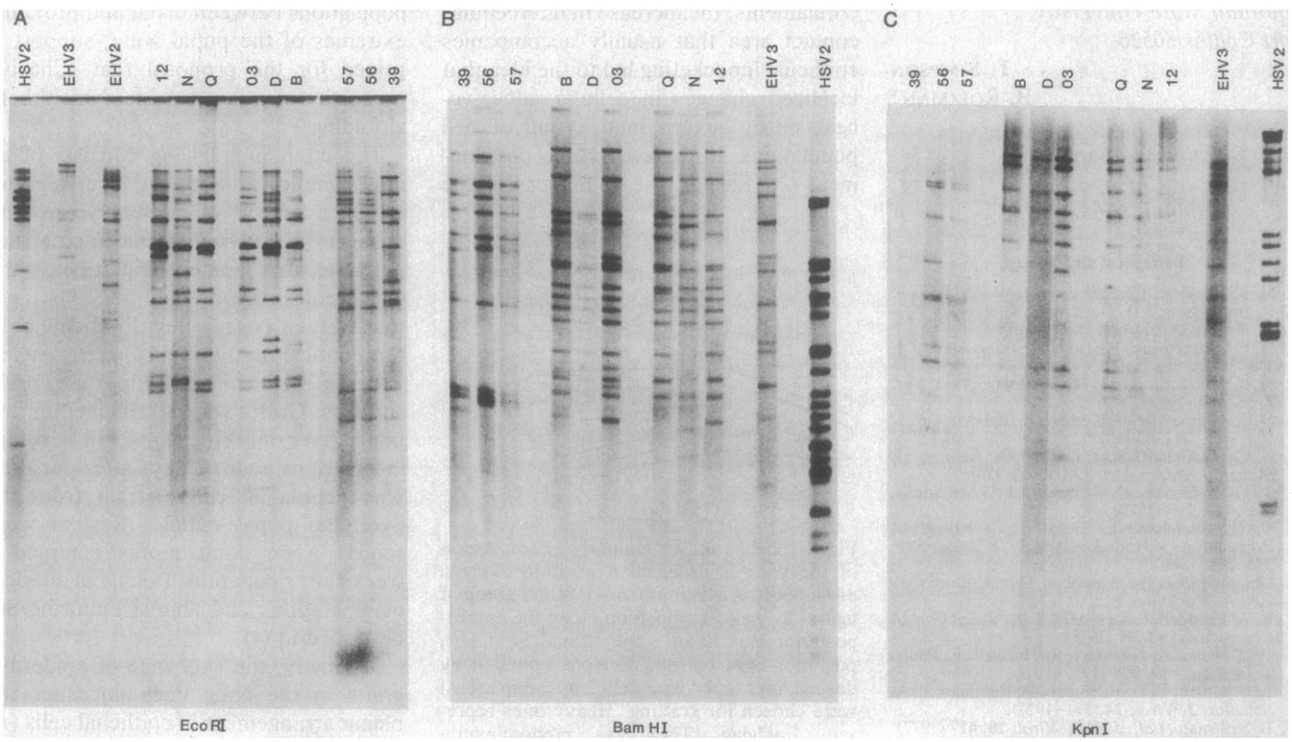

Fig. 1. Electrophoretically separated fragments of equine herpesvirus DNA's digested with (A) Eco RI, (B) Bam HI, and (C) Kpn I restriction endonucleases; *HSV2*, herpes simplex virus 2 DNA used as a marker.

the basis of this precedent, the F and R groups of equine herpesvirus should be distinguished taxonomically. We propose that DNA restriction endonuclease patterns of the F viruses be designated as prototypic of equine herpesvirus 1, whereas those of the R viruses be designated as prototypic of equine herpesvirus 4, in accordance with the recommendations for naming new herpesviruses by the Herpesvirus Study Group of the International Committee for Taxonomy of Viruses (*13*).

Our studies demonstrate the segregation of related viruses of the same host into distinct species on the basis of restriction endonuclease analyses of their DNA's. The emphasis on the DNA fragment patterns exemplified by the viruses analyzed in this study as the basis for classification reflects our prediction that F viruses may occasionally cause respiratory illnesses and that, conversely, R viruses may occasionally be responsible for sporadic abortions, in a manner analogous to the overlap in the human disease patterns and sites of isolation of herpes simplex viruses 1 and 2. The segregation of these viruses into distinct groups strengthens the previous reports suggesting biologic and possibly immunologic differences between R and F viruses and indicates that they should be treated as epidemiologically and taxonomically distinct infectious agents.

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Induction of Invagination in Insect Epithelium: Paradigm for Embryonic Invagination

Abstract. *The proposal that adhesive disparities between inpocketing populations of cells and surrounding epithelia drive epithelial invagination was tested in grafting experiments with moth pupal wing epithelium. Evidence exists that a cellular adhesiveness gradient spans the proximodistal axis of the wing. Although pupal wing cells normally do not invaginate or evaginate, epithelial folding can be induced after exchange of grafts from opposite ends of the proximodistal axis. The hypothesis that cytoskeletal elements are the primary agents in epithelial invagination should be reevaluated.*

Embryonic form is molded as epithelial cell sheets repeatedly fold inward and new cell arrangements arise. Initial inpocketing of epithelia is characterized by a localized thickening of the cell sheet (placode formation) that entails epithelial cell elongation and an increase in lateral contacts of the cells. Inpocketing of this thickened placode proceeds as epithelial cells broaden at their basal poles and assume wedge-shaped forms (*1*). Although the prevailing view attributes changes in cell shape to microtubule elongation and contraction of apical microfilaments, the increase in intercellular contact area that usually accompanies epithelial inpocketing led to the idea that localized changes in cellular adhesiveness might govern inpocketing of cell populations during embryonic development (*2*). Ideally, the soundness of the latter idea would be tested by exchanging small populations of cells in an undifferentiated tissue whose various regions differ quantitatively in adhesive properties. In insect epithelia, gradients of cellular adhesiveness appear to exist in single tissues (*3*, *4*). Numerous grafting experiments support the idea that a proximodistal gradient of cell adhesiveness spans the pupal wing epithelium of *Manduca*, the proximal cells of the wing being more adhesive than the distal cells (*4*). Since either invagination or evagination can result after exchange of cell populations between distal and proximal extremes of the pupal wing, support is gained for the proposal that adhesive properties of cells participate in epithelial folding.

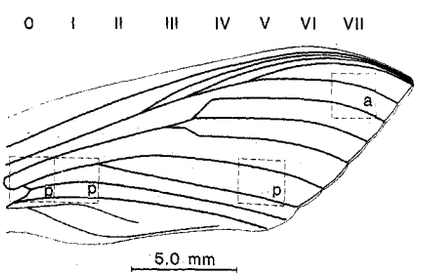

Fig. 1. Pupal wing of *Manduca*. Each region of the wing is assigned a position along a proximodistal axis (numerals *0* to *VII* at top of figure) as well as a position along the anteroposterior axis (*a* or *p*). Proximal is at left; posterior is at bottom. Regions bounded by dashed lines (approximately 2 by 2 mm areas) were chosen for grafting. Heavy lines represent tracheae. They are predominantly aligned along the wing's proximodistal axis and were used as landmarks during grafting.

The moth pupal wing consists of an upper and lower epithelial layer separated by a matrix of proteoglycans and collagen (*5*, *6*). Nuclei and intercellular junctions are located primarily at the apical poles of cells, and the cells taper toward their basal poles. Epithelial cells form cuticle on their apical surfaces and a basement membrane on their basal surfaces. Grafts were exchanged on the upper layer only (Fig. 1). Adult development begins about 2 days after pupation when epithelial cells retract from the overlying pupal cuticle. Results were scored when adult moths emerged 3 weeks after pupation. Details of surgical manipulations and animal culturing are described in (*4*).

Normally, the exchange of epidermal grafts in the wing does not alter the planar arrangement of epithelial cells (*4*). However, grafts (2 by 2 mm) exchanged between proximal (supposedly more ad-

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