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Serologic Differences in Strains of Herpes Simplex Virus

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Previous serologic studies have yielded conflicting evidence on the occurrence of antigenic differences among strains of herpes simplex virus. In some cross-neutralization tests in mice (*1*) and in mouse and chick embryos (*2*), antigenic differences were considered to have been demonstrated. No serologic differences were noted, however, in the strains examined in other observations involving neutralization in chick embryos (*3, 4*) and in infant mice (*5*) or employing cross-complement fixation (*4, 6*). In none of these experiments did the number of strains tested exceed four.

Current opinion appears to favor the belief that strains of herpes virus are remarkably uniform in their serologic behavior. Nevertheless, puzzling immunologic behavior has been observed occasionally (*7, 8*), suggesting that antigenic dissimilarity of herpes strains may indeed exist.

Table 1. Cross-complement fixation titers with six strains of herpes simplex and their antiserums.

Antiserum vs.	Antigens					
	AS	AH	O'C	HF	RH	WG
AS	8*	4	8	0	0	0
AH	16	16	16	4	0	8
O'C	16	16	64	8	8	8
HF	8	8	8	8	0	4
RH	4	8	8	4	32	32
WG	0	0	4	0	8	32

* Reciprocal of serum titer.

In order to examine the latter possibility, immune serums were obtained from rabbits that survived corneal infection with 11 strains of herpes virus that had been adapted to growth in the chick embryo. Nine of these were adapted to the chick embryo following isolation in eggs and two (HF and O'C) were laboratory strains previously passed in rabbits or mice. All showed the usual biologic properties of herpes simplex virus, and all but three produced symptomatic encephalitis in the rabbit.

Cross-complement fixation tests have been carried out between six of these viruses and their antiserums. All antiserums were tested simultaneously against a single antigen. Antigens consisted of bacteriologically sterile amniotic fluids from chick embryos dying after yolk-sac inoculation. Their optimal dilution was determined by a grid titration (antigen dilutions versus serum dilutions) with the homologous antiserum. Two minimal hemolytic units of complement were used in the tests, which were completed after 18-hr over-night fixation at 4°C by the addition of sensitized cells. The end-point chosen was 3+ fixation (50 percent hemolysis). The usual controls for specificity of the reactions were carried out simultaneously with the tests.

The titers obtained in the cross serologic examination of the six strains are presented in Table 1. A calculation of 2-titer ratios (*9–11*) yields the values appearing in Table 2. A ratio of 50-percent similarity in strains (ratio ½) or greater indicates relative serologic identity of strains, and a relationship of less than 50 percent (ratio $< \frac{1}{2}$) is required for demonstration of serologic differences. It is evident that, even in this limited group of strains, striking antigenic differences obtain between the closely related strains AS and AH on the one hand and the closely related strains RH and WG on the other. The other two strains are intermediate in their relationships to these extremes. Recent studies by Jawetz *et al.* (*12*) support the view that antigenic dissimilarity in herpes strains has been clearly demonstrated.

Additional observations on the relationship of these strains to others are being carried out by cross-complement fixation tests, and cross-neutralization tests along similar lines are in progress.

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Table 2. Cross antigenic relationships of six strains of herpes simplex. First figures refer to 2-titer antigenic ratios. Values in parentheses are percentages of relationship between strains.

	AS	AH	O'C	HF	RH	WG
AS	1 (100)					
AH	$\frac{1}{1.4}$ (71.4)	1 (100)				
O'C	$\frac{1}{2}$ (50.0)	$\frac{1}{2}$ (50.0)	1 (100)			
HF	$\frac{1}{2.8}$ (35.7)	$\frac{1}{2}$ (50.0)	$\frac{1}{2.8}$ (35.7)	1 (100)		
RH	$\frac{1}{8}$ (12.5)	$\frac{1}{8}$ (12.5)	$\frac{1}{5.7}$ (17.5)	$\frac{1}{8}$ (12.5)	1 (100)	
WG	$\frac{1}{16}$ (6.3)	$\frac{1}{8}$ (12.5)	$\frac{1}{8}$ (12.5)	$\frac{1}{8}$ (12.5)	$\frac{1}{2}$ (50.0)	1 (100)

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Nonmarine Miocene Arthropods from California*

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An excellently preserved fauna consisting mostly of fossil insects, arachnids, and branchiopods has been discovered in lacustrine deposits of middle Miocene or older age in the Calico Mountains, Mojave Desert, California. The composition of the fauna secured to date is shown in Table 1. Most of the specimens except the dragonfly nymphs, which are 18 mm long, have a maximum dimension of less than 3 mm.

The insects are preserved in petroliferous limestone nodules associated with bedded borates. At least three modes of preservation have been observed.

The dragonfly nymphs are preserved in calcite, the remainder of the specimens are preserved either in silica or in apparently only slightly altered organic material. Most of the specimens are uncrushed, and the quality of the preservation is such that hairs on the body and appendages are preserved. Figure 1 illustrates some of the detailed characteristics that are preserved.

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Fig. 1. Ventral view of a silicified adult midge. (×20)

The dragonfly nymphs were almost completely enclosed within limestone nodules when found. They have been excavated with a fine chisel-edged needle. The remainder of the specimens have been obtained from insoluble residues of limestone nodules digested in formic acid. Neither the organic nor the siliceous material appears to be affected by this acid. Most of the specimens are completely free of enclosing sediment and may be treated for study in much the same way as modern specimens.

Calcareous nodules in other fresh-water lake deposits of the Tertiary period should be examined for insect material. If additional specimens found at other localities are comparable in quality to those already discovered, the relatively unexploited field of stratigraphic paleoentomology may become a valuable tool in the study of the Tertiary lake deposits of western United States. In cooperation with entomologists of the U.S. Department of Agriculture and some universities, we are presently engaged in the study and description of the geologic and biologic implications of the insect fauna outlined in this paper.

The locality from which the insects have been collected is in the southeastern Calico Mountains about 5 mi north of Yermo and 1500 ft east of the Mule Canyon road, in the NE¼ NW¼ sec. 25, T. 10 N., R. 1 E., San Bernardino Meridian, Mojave Desert, California.

The geology of the Calico Mountains has been mapped recently by Thane H. McCulloh of the U.S. Geological Survey. He has divided the Tertiary section in the vicinity of the insect beds into four major lithic units:

4) Fluviatile and lacustrine sandstone, siltstone, mudstone, limestone, and conglomerate (1000+ ft).

3) Massive resistant hornblende andesite flow breccia (0 to 500 ft).

2) Thin- to medium-bedded lacustrine sandstone, siltstone, and shale containing numerous limestone beds in the lower half and gypsum, howlite, and colemanite beds locally near the top (900 to 1100 ft); the fossil insects are found near the top of this unit.

Table 1. Composition of the fauna.

Kind of fossil	Aquatic	Non-aquatic	Approx. No. of specimens	Approx. % of fauna
May-fly nymph	x		1	< 1
Dragonfly nymph	x		4	1
Thrips		x	17	5
True bug (3 species)		x	4	1
Beetle larva	x		130	30
Midge (2 species)			100	25
Larva	x		(23)	(6)
Pupa	x		(55)	(15)
Adults		x	(14)	(4)
Mite (2 species)		x	6	> 1
Spider		x	2	< 1
Fairy shrimp	x		150+	35