perature ranges is quite different. Besides providing a means of extrapolating the expected life of virus at —62° storage, Fig. 1 also indicates an activation energy of 14 K cal/mole for inactivation of the virus. This is lower than all of those reviewed by Woese (2) for animal viruses, being comparable only with the values 18 K cal/mole listed for the low temperature inactivation of measles virus.

Conclusions. Vaccinia virus is exceedingly stable, with an indicated half-life of many years at —70°C. It may lose virtually all infectivity (PFU) in the temperature range 56° to —20°C without loss in countable particles. In the range 37°C to —20°C the activation energy for the process is 14 K cal/mole, a value which is lower than that of most viruses and much lower than that for the reaction at 56°C. Apparently the process of degradation of the virus is quite different in the 2 temperature regions.

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Relationship Between the Envelope and the Infectivity of Herpes Simplex Virus.* (29045)

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Pirie(1) raised the question of whether the herpes virus particle requires an envelope in order to be infective. Wildy *et al*(2), using the negative staining technique, clearly differentiated between 2 morphological types of herpes virus: the naked particles (possessing cubic symmetry) and enveloped particles. Recently Watson and Wildy(3) suggested that the envelope is *not* essential for infectivity, although data to support this view were not presented. Holmes and Watson(4) found that enveloped particles are more readily absorbed to cells than are naked particles. We observed the same phenomenon, which suggested to us that enveloped particles might be associated with infectivity. Brief ether treatment removes enveloped particles from aqueous suspension, drastically reduces the infectious titer, but does not noticeably alter the fine structure of naked particles(5). Although suggestive, none of the above experiments clearly answers whether or not naked particles are infectious.

Materials and methods. A physical separation of naked from enveloped particles was attempted, using the method described by Roizman and Roane(6) for fractionating particles on the basis of differences in their bouyant densities in cesium chloride density gradients. Freshly grown virus was sonically treated briefly(5), centrifuged at 2,000 × *g* for 5 minutes to remove large viral aggregates, and the supernatant suspensions layered over 4.0 ml cesium chloride having a density of 1.34. Tubes were centrifuged for 24 hours at 40,000 rpm and fractionated by the bottom drip method. Plaque titrations,

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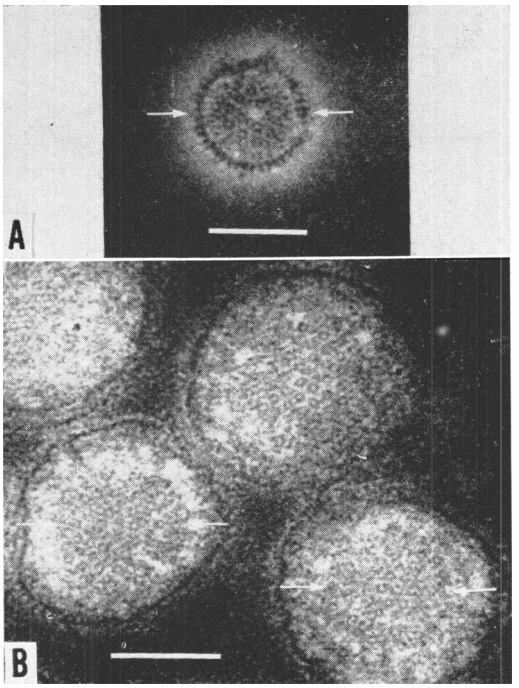

FIG. 1. A. Naked form of herpes virus. B. Enveloped form of herpes virus negatively stained with potassium phosphotungstate at pH 7.3 (bar equals 100 mμ).

virus staining and virus particle counts were done by methods described previously(5). Particles in the electron micrographs were counted and classified as either naked or enveloped. Naked and enveloped forms are illustrated in Fig. 1A and B. The results obtained with 2 herpes virus strains are reported here: the HSV XIII strain, kindly provided by Dr. Fred Rapp of this laboratory(7) and the SOK strain, a first passage of a recent isolate from a herpetic lip lesion. The latter strain is described because of its unusually low particle:PFU ratio.

Results. Table I shows the results of 2 experiments in which the 2 strains were fractionated, titrated and the particles counted. In the first experiment it is seen that fractions 4 and 5 contained less than 1% enveloped forms (none were seen in over 200 particles examined in each preparation), and less than one thousandth of the total infectious units. The particle:PFU ratios of these 2 virus fractions were extremely high, over 10^5 and 10^4, respectively. This is in sharp contrast to the next 3 fractions, which contained 88-92% enveloped forms, were highly infectious and had relatively low particle:PFU ratios (69-81).

Similar results were obtained with the SOK strain and these are shown in the lower part of Table I. Fraction 5, containing >99% naked particles, had a particle:PFU ratio of 8,000. This is in sharp contrast to the highly infectious fractions 8-10, which contained large proportions of enveloped particles (70-82%) and had a particle:PFU ratio of 10-12. Fraction 7 contained a mixture of the 2 types, naked particles predominating (82%). It seems clear, under the conditions of these experiments, that fractions containing *only*

TABLE I. Density Gradient Fractionation of Herpes Simplex Virus.

	Fraction No.*	No. physical particles/ml (× 10^7)	No. PFU/ml (× 10^7)	Particle: PFU ratio	% enveloped forms
Exp #1 Strain HSV-XIII	8	170	2.1	81	92
	7	180	2.6	69	91
	6	110	1.5	74	88†
	5	60	.005	12,000	<1
	4	110	<.001	>100,000	<1
	3	<1	<.001	—	—
Exp #2 Strain SOK	10	140	14	10	70
	9	220	18	12	72
	8	500	41	12	82
	7	250	27	9	18†
	6	110	.1	1,100	<1
	5	24	.003	8,000	<1

* Eleven fractions were collected in each experiment; lower numbered fractions were taken from the lower part of the tube by the drip method and were therefore more dense.

† Most of the naked particles in this fraction and those following it were coreless, as judged by both positive and negative staining.

naked herpes virus particles are practically non-infectious. It is seen in both experiments (with 2 different strains) that there was about a 1000-fold difference between the particle:PFU ratio of the lowest (naked particle) fractions and the less dense upper fractions, which contained large proportions of enveloped particles. It should be noted that a high proportion of the naked particles in the first fractions of both of these experiments contained morphologically complete or nearly complete DNA cores. The naked particles contaminating the enveloped fractions were mostly coreless capsids. Their presence in the upper fractions might be expected because their low content of nucleic acid makes them less dense.

The envelope of herpes virus has been shown to be derived from and to consist mainly of host cell material(3). Determining the mechanism by which such an envelope can contribute so markedly to the infective potential of the naked herpes virus particle seems to be a matter of considerable importance in the understanding of the biology of this virus. Myxoviruses possess such envelopes, which are derived in much the same way(8,9) and have structures similar to the envelopes of herpes virus(3). Herpes viruses are unique in that they are the only group of cubically symmetrical viruses known which possess an envelope derived from the plasma membrane.

It has been pointed out that enveloped particles are adsorbed to cells preferentially, but Holmes and Watson(4) have shown that some naked particles do adsorb to susceptible cells. Simple inability to adsorb, therefore, cannot account for the observed non-infectivity of naked herpes virus particles. The relatively large holes in the capsomeres of the herpes virus capsid (40 Å) may permit the access of destructive enzymes or other elements into the inner structure of the naked particle. It is possible that the envelope helps stabilize the cubically symmetrical particle against rapid inactivation by serving as a physical barrier to destructive organic molecules such as nucleases, proteolytic enzymes, or to other factors which could cause inactivation. Conversely, it is possible that the envelope slows the leakage of essential components associated with the biological stability of the inner particle.

Summary. Crude herpes virus suspensions were fractionated by the cesium chloride density gradient sedimentation method into enveloped and naked virus particles. Particles without envelopes were found to be almost non-infectious, whereas fractions containing enveloped particles were highly infectious. It appears, therefore, that the envelope plays an essential role in herpes virus infection.

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