THE INFLUENCE OF pH AND OSMOLALITY ON FIXATION OF THE FOWLPOX VIRUS CORE

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INTRODUCTION

It has generally been accepted during recent years that the fixatives of choice for delicate biological material should have a pH and osmolality in the same range as the fluids of the tissue under study (9). With rare exceptions, such as certain specially adapted sea organisms, biological tissues usually are fixed for electron microscopy in solutions of 300–350 milliosmols, and pH 7.0–7.6. Relatively little regard has been given to the fixation of viruses; rather, it has been assumed that the best conditions for virus fixation coincide with those which yield good preservation of the host tissue.

It was shown by Peters and coworkers that the DNA-containing cores of vaccinia and other poxviruses possess an ordered, complex substructure, usually characterized by a triplet of tubular elements (4–6). Similar structures in fowlpox virus (FP) could not be demonstrated (1), and it was because of this that an investigation of the effects of variations in pH and osmolality was undertaken. It is the purpose of the present report to demonstrate the sensitivity of fowlpox virus to variations in pH and osmolality, and to show that highly ordered substructure of the core not visible by standard methods is revealed after fixation under unconventional conditions.

MATERIALS AND METHODS

The extremes of pH and osmolality were selected arbitrarily as pH 2–9, and 100–2000 milliosmols, respectively, the latter adjusted either with NaCl or sucrose. For this purpose the work of Maser et al. (3) was a useful reference, but higher osmolalities were determined by freezing-point depression (Fig. 1). The strain of virus and the method of infecting chick scalps have been described (7). Fixation of scalps was carried out in 0.5–2% glutaraldehyde, buffered or unbuffered, from 2 to 18 hr at 4°C, followed by a 2 hr rinse in a solution of the appropriate pH and osmolality, but without fixative. Embedding was done in Epon (2), and staining was accomplished with a saturated solution of uranyl acetate in 70% methanol, followed by lead citrate (8).

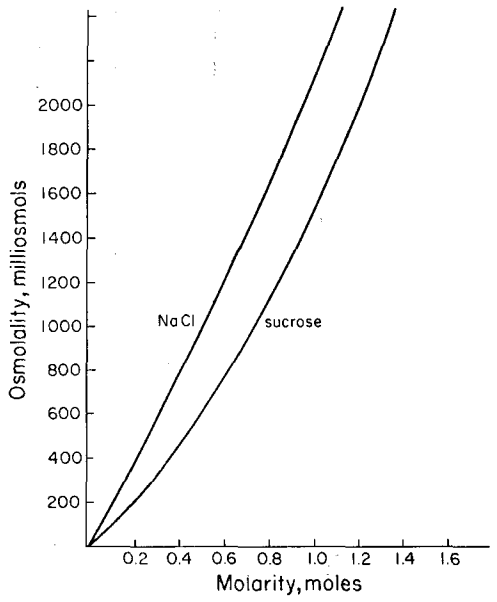
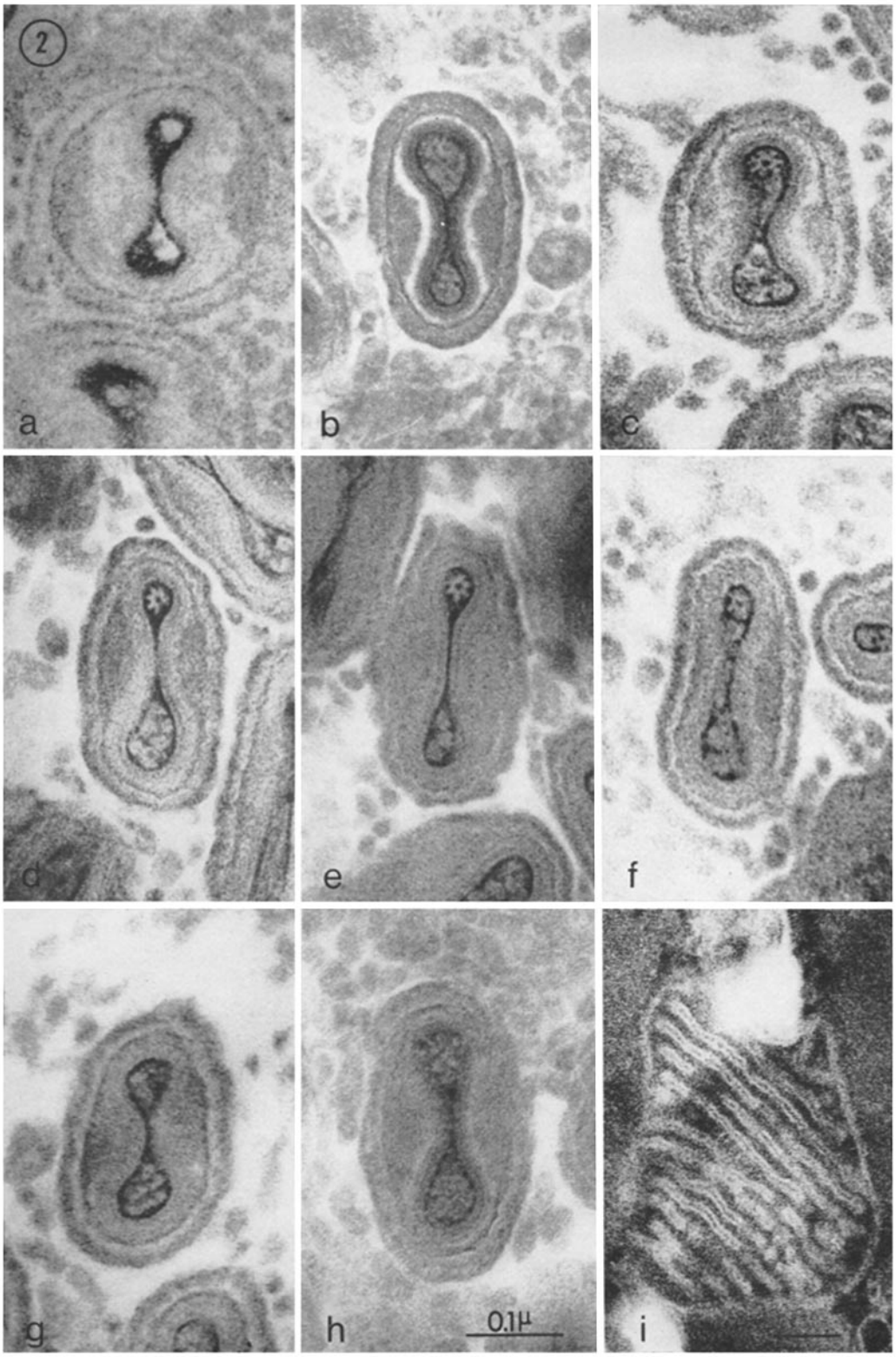


FIGURE 1 Osmolality of sucrose and NaCl expressed as a function of molar concentration.



RESULTS

The most striking effects of low osmolality (Fig. 2 *a*) were rounding of the virus and concomitant separation of outer components from the core, and the occurrence of hollow spaces within the core. At standard conditions, pH 7 and 350 milliosmols, these changes were much less pronounced, but regular fine structure within the core was still not clearly visible (Fig. 2 *b*). It was established that high osmolality and low pH are desirable conditions for fixation of the FP core, as evidenced by the emergence of an intriguing structure, designated the "Blümchen," at pH 3 and 500 milliosmols (Fig. 2 *c*). At pH 3 and 700 milliosmols, not only the Blümchen became visible, but an array of smaller units as well (Fig. 2 *d*). The appearance of these structures was not significantly altered by osmolalities as high as 2000 (Fig. 2 *e*). For this extreme of osmolality, high concentrations of NaCl proved to be deleterious to the nucleoprotein core (Fig. 2 *f*), a problem that was circumvented by the use of sucrose to achieve high osmolality. pH 3 was found to be optimum. Below this value (Fig. 2 *g*) the nucleoprotein of the core appeared somewhat clumped, and at alkaline pH values severe loss of structural detail within the core occurred (Fig. 2 *h*). The concentration of glutaraldehyde was eventually lowered to 0.5%, resulting in an improvement in contrast. In addition, several different buffer systems were tested, but were found to be totally unnecessary. It therefore was concluded that an optimum fixative for the fowlpox virus core is 0.5% unbuffered glutaraldehyde at pH 3 and 700 milliosmols. The results of these studies are summarized in Table I.

TABLE I

Summary of the Effects of pH and Osmolality on Preservation of the Fowlpox Virus Core

	Milliosmols			
pH	300	500	700	1000
9	−	−	−	−
7	−	−	+−	+−
5	−	−	+	+
3	−	+	+++	++
2	−	−	+	+

+, Blümchen clearly visible; +++, Blümchen and small units of the core clearly visible.

Fixation at high osmolality has the additional advantage that the general condition of the cell as reflected by its osmotic capability can be recognized quickly during scanning at low magnification (Fig. 3). The intact cells, in which the highest quality virus is found, are shrunken and dense, with reversely contrasted membranes and well preserved mitochondria (Fig. 2 *i*).

DISCUSSION

The structure of the FP core is comparable to that of the core of other poxviruses (4–6), but the

FIGURES 2 *a–i* Effects of fixation under various conditions of osmolality and pH on the preservation of the fowlpox virus core. Osmolality was adjusted with sucrose unless otherwise stated. (*a*) pH 3; 200 milliosmols. There is a marked rounding of the virus, separation of outer components, and presence of hollow spaces within the core. (*b*) pH 7; 350 milliosmols. Separation of outer components and lateral bodies from the core still occurs, and there is only faint evidence (top-left) of regular fine structure in the core. (*c*) pH 3; 500 milliosmols. There is some separation of outer components from the core, but the highly ordered Blümchen is clearly visible inside of the core (top). (*d*) pH 3; 700 milliosmols. Optimum preservation of the virus. Note Blümchen at one end of the core and six smaller units at the other end. (*e*) pH 3; 2000 milliosmols. The core is well preserved even at this extreme of osmolality. (*f*) pH 3; 2000 milliosmols adjusted with NaCl. NaCl in high concentration causes apparent dissolution of DNA from areas within the core. Note remainder of Blümchen (top). (*g*) pH 2; 700 milliosmols. Fine structure of the core is indistinct at very acid pH, but indications of the Blümchen are still present (top-right). (*h*) pH 9; 700 milliosmols. Severe alterations in the DNA matrix of the core occur at alkaline pH. (*i*) pH 3; 2000 milliosmols. Portion of a mitochondrion showing good preservation and strong reversal of contrast typical after fixation at high osmolality. *a–h*, × 150,000; *i*, × 100,000.

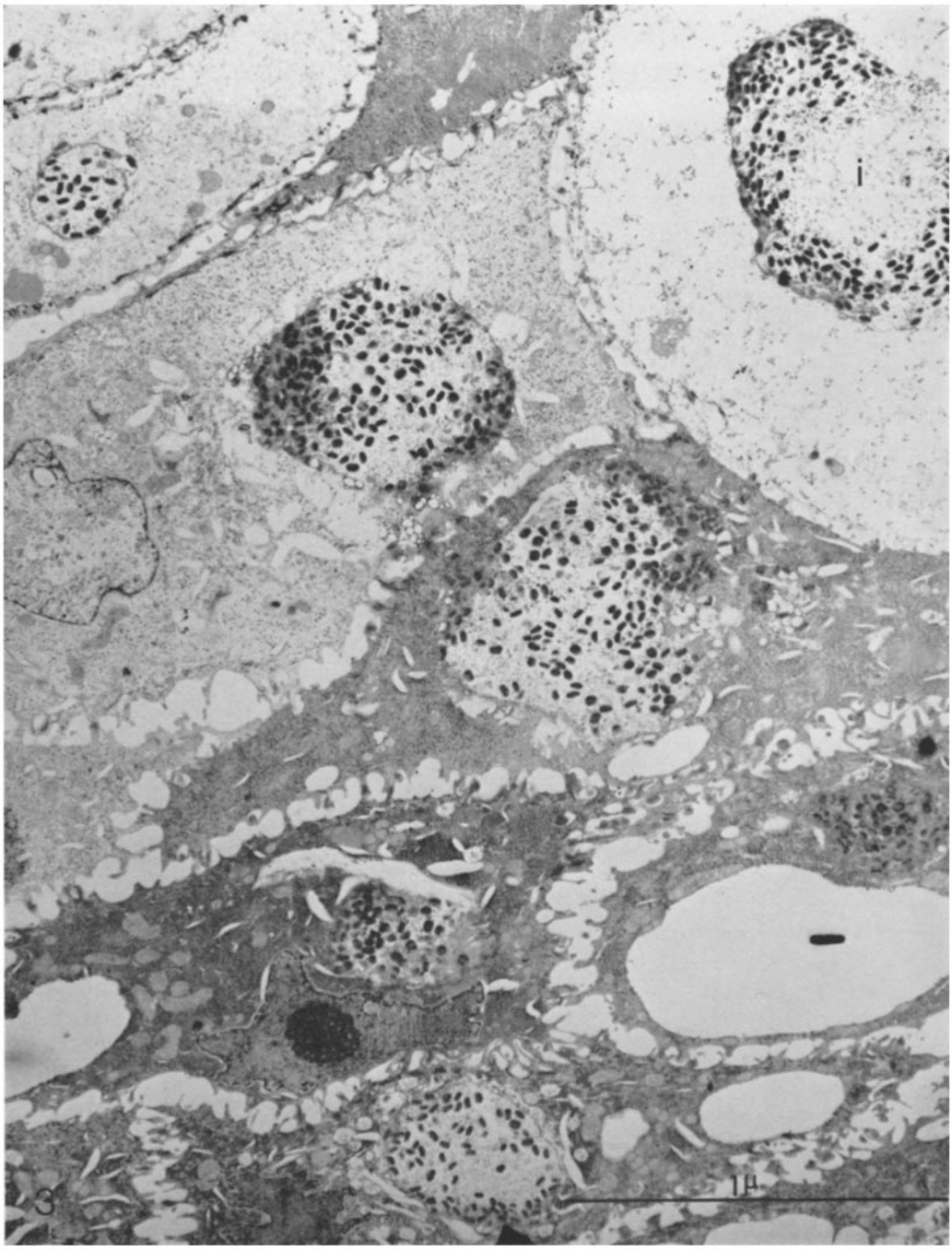


FIGURE 3 Fowlpox virus-infected chick scalp illustrating relative condition of the cells by comparison of density after fixation at high osmolality. Cells of lower density are in various stages of degeneration, while shrunken cells of higher density are still osmotically intact. (*i*), inclusion body containing fowlpox virus. Fixation: 0.5% glutaraldehyde at pH 3; 2000 milliosmols. × 5500.

Blümchen appears to be unique to this virus. It should be emphasized that some evidence of the Blümchen has been seen in preparations fixed by generally unfavorable methods (Fig. 2 *f*, *g*), and also after fixation under standard conditions (Fig. 2 *b*). This indicates that this unusual structure, best seen in tissue samples fixed at pH 3 and 700 milliosmols, is not merely an alteration of viral core structure produced by low pH and high osmolality, but instead is an actual component of the nucleoprotein complement of fowlpox virus.

The results suggest that certain virus substructures, and perhaps even cellular substructures, may be clearly visualized in ultrathin section only after fixation by unconventional methods. It is suggested further that studies of specific fine structures by electron microscopy should be preceded by a thorough investigation of fixatives, particularly with regard to osmolality and pH. Further results concerning the fine structure of the Blümchen and the FP core are being prepared for publication.

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