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NOTES

Effect of Altered Osmotic Pressure on the Growth of Sindbis Virus

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Since reducing the osmotic pressure of the medium lowers the yield of influenza virus (M. Eaton and R. Scala, Proc. Soc. Exptl. Biol. Med. **92**:289, 1956) and polio virus (E. A. Tolskaya, V. I. Agol, M. K. Vorishilova, and G. Y. Lipskaya, Virology **29**:613, 1966), we hoped that a study of the effect of osmotic pressure on the growth of Sindbis virus would contribute to better understanding of arboviruses. We found that hypotonic medium apparently affects a terminal step in Sindbis virus maturation.

Methods for virus growth and titration have been described (E. R. Pfefferkorn and H. S. Hunter, Virology **20**:433, 1963); the osmotic pressure of the medium was adjusted by varying the amount of NaCl, and was recorded as the percentage of normal. We measured the synthesis of virus-specific ribonucleic acid (RNA) by incorporation of ^{3}H-uridine in the presence of 1 μg of actinomycin D per ml.

Figure 1 shows that altering the osmotic pressure of the medium reduced the virus yield. High osmotic pressures inhibited viral RNA synthesis and growth to the same extent. At low osmotic pressures, these two parameters were clearly dissociated: at 60% osmotic pressure, the virus yield was reduced from 8,000 to 15 plaque-forming units per cell, while the synthesis of cell-associated, virus-specific RNA was greater than the control. Release of viral RNA in the form of virions cannot account for this difference since, even in normal infection, most of the virus-specific RNA remains cell-associated (*unpublished data*).

Preliminary experiments have shown that the viral RNA synthesized at 60% osmotic pressure is infectious; thus, a late function in viral growth must be sensitive to low osmotic pressure. To localize this function, monolayers incubated at 60% osmotic pressure for various periods of time after infection were placed in normal medium, and virus production was observed (Fig. 2). Control cultures, incubated in normal medium throughout, released virus at a linear rate beginning 3 hr after infection. The culture shifted to normal medium at 4 hr rapidly began to produce virus at a rate similar to that of the control, whereas those shifted later appeared to produce virus at an accel-

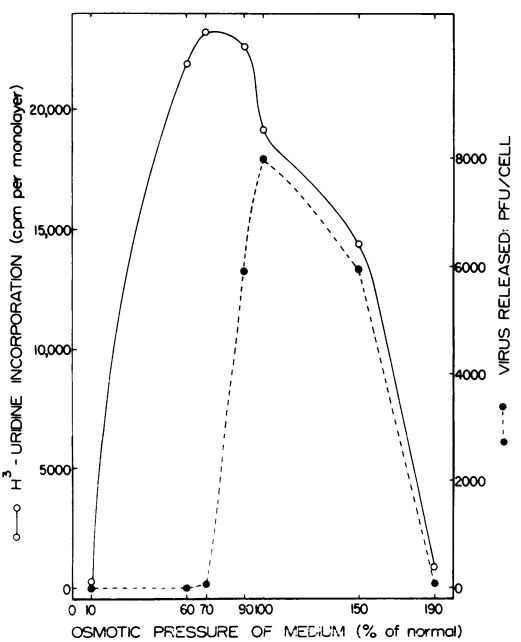

FIG. 1. *Effect of media of various osmotic pressures on viral growth and RNA synthesis. After virus adsorption from Hanks' balanced salt solution of normal osmotic pressure, triplicate monolayers were incubated in media of the indicated osmotic pressure in the continuous presence of actinomycin D (1 μg/ml). After 3 hr, the medium was replaced by fresh, prewarmed medium containing ^{3}H-uridine (0.5 μc/ml, 20 mc/μmole). At 7 hr, the media were saved for virus titration, and the monolayers were processed to determine trichloroacetic acid-precipitable radioactivity.*

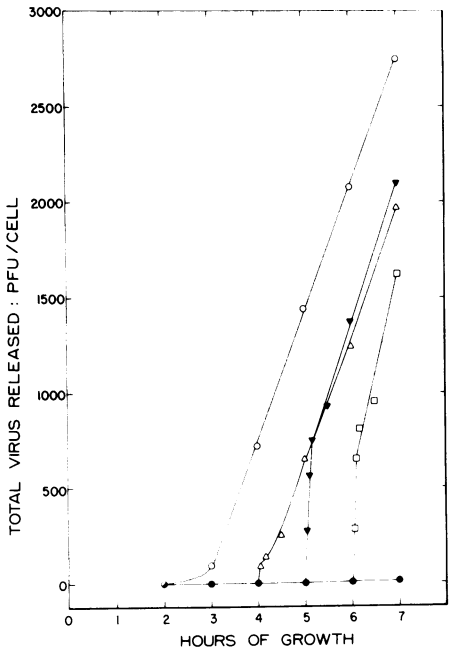

FIG. 2. *Virus growth in normal and hypotonic media; effect of shifting to normal after various periods of incubation at low osmotic pressure. After infection, 60% osmotic pressure medium was added to all cultures except the normal control. At 1 hr after infection, all media were changed to remove unadsorbed virus. At each point indicated on the graph, the medium was poured off and saved for titration; fresh, prewarmed medium of the proper osmotic pressure was added. Symbols: ○, 100% osmotic pressure medium throughout; ●, 60% osmotic pressure medium throughout; △, shifted from 60 to 100% medium at 4 hr after infection; ▼, shifted from 60 to 100% medium at 5 hr; □, shifted from 60 to 100% medium at 6 hr.*

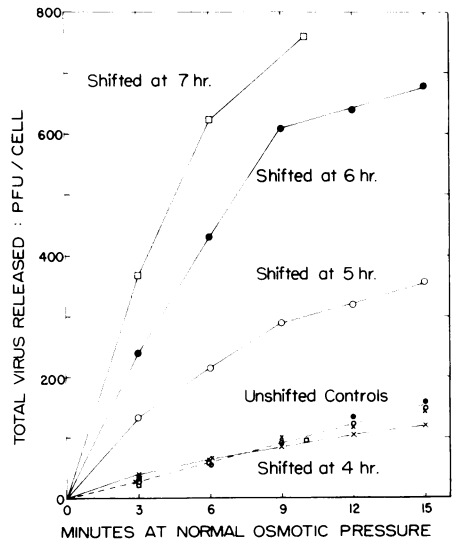

FIG. 3. *Virus production after shift from low to normal osmotic pressure medium. Infected monolayers were incubated in 60% osmotic pressure medium until shifted to normal medium at the time indicated. To remove previously synthesized virus, the medium was changed four times during the 15 min prior to the shift. At the indicated time, the hypotonic medium was replaced by normal medium, and virus production was followed by replacing the medium at the indicated intervals and saving samples for titration. A control monolayer, similarly treated but incubated at normal osmotic pressure throughout, was included at each time interval.*

erated rate that nearly permitted them to catch up with the control.

We further examined the period of accelerated virus release by changing the medium every 3 min after shift to normal osmotic pressure (Fig. 3). The culture incubated for 4 hr at 60% osmotic pressure before shifting to normal medium was not significantly different from the controls. Those shifted later exhibited an immediate burst of virus production: the longer the incubation at low osmotic pressure, the more virus released. The rapidity with which the total number of virions released approached control levels after reversal of the inhibition suggests that only a terminal step in virus production may be inhibited. This sensitive step is probably part of the process by which the viral nucleocapsid buds through the cytoplasmic membrane, although it could occur earlier.

Low osmotic pressure probably inhibits a different function in Sindbis virus and polio virus infections, for the latter inhibition is reversed only after an 0.5-hr lag (E. A. Tolskaya et al., Virology **29**:613, 1966). Electron microscopy should help to define the sensitive event in Sindbis virus growth. It will also be interesting to learn whether the phase of accelerated virus production that follows shift to normal osmotic pressure requires new protein synthesis or a source of energy.

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