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Rate of Inactivation of Equine Encephalomyelitis Virus (Eastern Strain) Relative to H ion Concentration.*

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Detailed studies of the pH stability range of infectivity of plant viruses, summarized recently by Stanley,[1] show that for each virus studied there is a well defined region where infectivity is relatively stable. On both sides of this region infectivity is lost in a regular way so that the curve plotted to relate infectivity with pH of the medium is smooth and trapezoidal. Certain of the animal viruses[2,3] appear to behave in a like manner. It was entirely unexpected, then, when conditions were encountered under which the virus of equine encephalomyelitis (Eastern Strain) seemed to behave in a different way.

The experiments were made on virus propagated in chick embryos.[4] Virus-infected embryos were "harvested" when moribund and ground with sand in hormone broth to a 10% suspension. Centrifuged free of sand and gross tissue particles, the whole extract was mixed with composite buffer solution (0.05M) of various pH's.[2,3] Mixtures were maintained between 0°C and 5°C, and after various intervals, usually 1 hour, 1 day, and 1 week, portions were removed for test. The pH of each was readjusted approximately to neutrality and virus infectivity determined by titrations in decimal dilutions in mice.[5] pH was determined and frequently checked with the glass electrode.

Four such experiments covering the range from pH 1.0 to pH 12.0 have shown essentially uniform results, and one is summarized in Fig. 1. The curve after 1 hour is similar in contour to those of other viruses, the region of greatest stability lying between pH 3.5 and pH 11.5. After 1 week, maximum stability is at pH 7.5 to

* This work was made possible through the interest and aid of Lederle Laboratories, Pearl River, N. Y.

1 Stanley, W. M., Harvey Lectures, 1937-1938.

2 Beard, J. W., and Wyckoff, R. W. G., *J. Biol. Chem.*, 1938, **123**, 461.

3 Beard, J. W., Finkelstein, H., and Wyckoff, R. W. G., *Science*, 1937, **86**, 331.

4 Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G., *Science*, 1938, **87**, 490.

5 Olitsky, P. K., and Cox, H. R., *J. Exp. Med.*, 1936, **63**, 311.

pH 8.5, while a second region of relative stability is apparent between pH 3.5 to pH 5.0. About midway between pH 5.0 and pH 6.5 inactivation is almost complete. The curve after 1 day is similar in shape, though the inverted peak between pH 5.0 and pH 6.5 is not so prominent as that after 1 week.

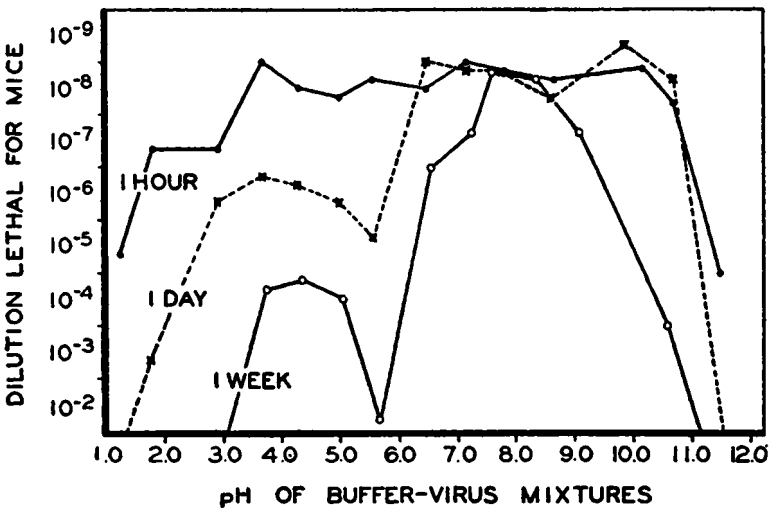

FIG. 1.

pH-activity curves for unpurified equine encephalomyelitis virus (Eastern Strain) in crude extract of chick embryo tissue in which virus was propagated.

The behavior of the virus in the limited region of rapid inactivation was examined in more detail by increasing the number of mixtures with smaller differences in pH between pH 3.0 and pH 6.5. It was found that, instead of a single restricted point, there was a narrow inverted plateau bounded by pH 5.1 and pH 5.7 where inactivation seemed at a maximum.

The possibility existed that this phenomenon might be associated with changes in the virus at its iso-electric point. The iso-electric point of the virus purified by ultracentrifugation,[6] however, determined with the Northrop-Kunitz microcataphoresis cell, was found to lie between pH 3.7 and pH 4.1, far below the region of rapid inactivation. This result was corroborated with the Todd macrocataphoresis apparatus. The purified, fully infectious virus still possessed a negative charge at pH 4.5.

An obvious factor to be considered is the presence of a substance,

[6] Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 771. The instrument employed was the Wyckoff quantity type and we are grateful to Mr. W. Hurst of the Department of Physics for his interest in its construction and maintenance.

possibly of the nature of an enzyme,[7] in the chick embryo tissue capable of inactivating the virus under optimum conditions of pH. Preliminary experiments with purified virus indicate that this may indeed be the case. With the purified virus free of chick tissue extract and hormone broth the region of rapid inactivation between pH 5.0 and pH 6.5 has not been apparent. Further experiments in which no hormone broth was employed have indicated that the inactivating material may be present also in normal chick embryo tissue. These possibilities are being investigated further.

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An Antidiuretic Effect Produced by Certain Preparations of Heparin.*

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In attempts to get evidence of the presence of an antidiuretic hormone in blood[1] it was found that commercial preparations of heparin produce antidiuresis when injected intravenously into diuretic rabbits in doses of 1 to 4 mg. Six preparations have been used in 62 injections. Three of 4 lots, obtained from the Connaught laboratories and derived from beef lung, contained 15 anticoagulant units per mg; the fourth, a highly purified solution (lot 43-1), obtained through the courtesy of Dr. C. H. Best, contained 1000 units per cc. Of 2 lots obtained from Hynson, Westcott and Dunning, both derived from dog liver, one contained 5 units per mg, the other 20 units per mg. All except lot 43-1 consistently produced brief definite antidiuresis (Fig. 1), characteristically preceded by a 10-minute latent period, when injected intravenously into rabbits. No effect was produced by subcutaneous injection into rats or frogs.

The antidiuretic action is not a property of the anticoagulant factor in the heparin, for the most potent Connaught specimen had little or no antidiuretic action and the effects of the 2 Hynson, Westcott and Dunning preparations were not proportional to their anticoagulant potencies.

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[7] Best, R. J., and Samuel, G., *Ann. Appl. Biol.*, 1936, **23**, 759.

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[1] Walker, A. M., *Am. J. Physiol.*, 1938, **123**, 210.