mate could be related to a renal action, experiments were performed using the conscious, trained beagle in which urinary electrolytes and renal clearances were measured. Electrolytes were not affected by intravenous doses of 1-90 mg/kg of meprobamate or 10-50 mg/kg, of mebutamate. Clearance of inulin and p-aminohippurate were measured using doses of 30 mg/kg of meprobamate and 20 mg/kg of mebutamate. Neither clearance was affected by meprobamate, and there was only a small reduction in PAH clearance following mebutamate administration. It was concluded that these compounds are without significant renal effects.

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## pH, Protein, and Semliki Forest Virus Infectivity.* (28616)

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An effect of pH and serum protein on the persistence of arbovirus infectivity has been known for many years(1,2). Loss of Semliki Forest virus (SFV) infectivity during attempts at purification led us to investigate the relationship between pH and stability of SFV in the presence and absence of protein. Persistence of SFV and Western equine encephalitis (WEE) virus infectivity in several tissue culture diluents was also studied and a possible stabilizing effect of amino acids noted.

*Materials and methods.* SFV and WEE were prepared as 10% W/V homogenates of infected mouse brain in sterile distilled water. After centrifugation for 20′ at 1500 *g* to sediment cellular debris, the supernate was frozen as 1 ml aliquots in sealed glass ampules and stored in a $CO_2$ (dry ice) box.

Crystalline bovine serum albumin purchased from Mann Research Laboratories had been prepared by electrophoretic separation in cellulose acetate. Tissue culture media were obtained from Microbiological Associates, Bethesda, Md.

The preparation of sodium phosphate buffer solutions, determination of pH, methods of dilution, incubation of serial dilutions, and mouse assay of infectivity were as described previously(3), with the exception that a thawed ampule of virus was immediately diluted 10-fold in chilled sterile distilled water to give a $10^{-2}$ dilution of infected mouse brain, instead of a $10^{-0}$ stock being diluted 100-fold. From the $10^{-2}$ dilution of virus, subsequent dilutions were made in one of the solutions under study using chilled diluents and glassware. One set of dilutions was immediately inoculated into groups of 5 male 18-24 g Swiss-Webster mice per dilution. The second set was incubated for 2 hours in a constant temperature water bath and then similarly assayed for mouse infectivity, as judged by lethality. SFV was assayed for infectivity by i.p. inoculation. WEE was assayed by i.c. inoculation because this strain was of low pathogenicity by other than i.c. injection. Mice were observed for 2 weeks

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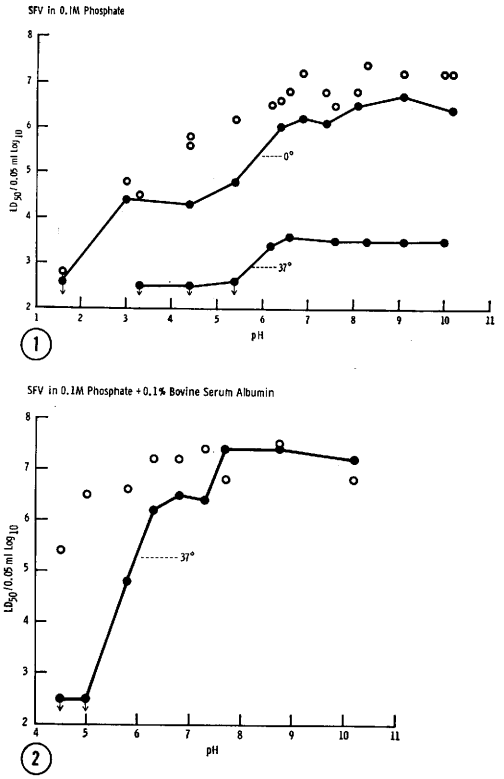


FIG. 1. Infectivity of SFV diluted in 0.1 M sodium phosphate buffer, before (○) and after (●) incubation of serial dilutions for 2 hr at 0° and 37°C.

FIG. 2. Infectivity of SFV diluted in 0.1 M sodium phosphate + 0.1% bovine serum albumin, before (○) and after (●) incubation for 2 hr at 37°C.

and deaths occurring from the second through the 14th day were used for calculation of the $LD_{50}$(4).

*Results. SFV in 0.1 M phosphate.* When SFV was serially diluted in chilled 0.1 M phosphate of varying pH and immediately inoculated i.p. in mice, the $LD_{50}$ from pH 6 to above pH 10 was consistently high (Fig. 1) and approximated that obtained when the virus was serially diluted in 5% rabbit serum in isotonic NaCl. Below pH 6 there was a decrease in infectivity with increasing acidity.

When such serial dilutions of SFV were kept for 2 hours in an ice water bath prior to mouse inoculation, there was a slight decrease in infectivity (Fig. 1). When serial dilutions of SFV were incubated at 37°C for 2 hours before inoculation, there was a marked loss of infectivity over the entire pH range studied (Fig. 1). No infectivity was demonstrable at $10^{-3}$ dilutions when the pH was below pH 6. Of interest was the preservation of infectivity at $10^{-3}$ dilutions between pH 6 and pH 10. Within this pH range mortality at $10^{-3}$ was 29/30, whereas mortality at $10^{-4}$ was only 1/30. Loss of infectivity at dilutions greater than $10^{-3}$ was not restricted to 0.1 M phosphate but was also observed when SFV was diluted in Gey's, Hanks' or Earle's solution, all complex, glucose-containing inorganic salt solutions used in tissue culture (Table I).

*SFV in 0.1 M phosphate + bsa.* When SFV was serially diluted in 0.1 M phosphate + 0.1% crystalline bovine serum albumin (bsa) and immediately inoculated i.p., the initial level of infectivity was like that in 0.1 M phosphate alone. However, after incubation of such serial dilutions at 37°C for 2 hours, a high level of infectivity, similar to that before incubation, persisted in diluents above pH 6; whereas in more acid solutions there was a marked loss of infectivity despite the presence of protein (Fig. 2). The addition of 0.01% bsa to 0.1 M phosphate resulted in erratic preservation of infectivity above pH 6, while addition of 1% bsa did not increase the persistence of infectivity below pH 6. Some other proteins were found

TABLE I. Infectivity, as Judged by Mouse Lethality, of Semliki Forest Virus and Western Equine Encephalitis Virus Serially Diluted in Several Tissue Culture Media and Incubated at 37°C for 2 Hours. Titer of SFV in the listed diluents before incubation at 37°C, as mean and range of $-\log_{10}$ i.p. $LD_{50}$/0.05 ml, was 6.5 ± 0.3. Initial titer of WEE, as $-\log_{10}$ i.c. $LD_{50}$/0.03 ml, was 8.4 ± 0.4. Figures in the table also refer to $-\log_{10}$ $LD_{50}$.

| Diluent | SFV, i.p. | WEE, i.c. |
|---|---|---|
| Gey's basal salt solution | 3.5 | 4.5 |
| Hanks' " " " | 3.5 | 4.4 |
| Earle's " " " | 3.4 | 4.5 |
| Medium 199 | 5.8 | 7.3 |
| Eagle's basal medium | 5.4 | 7.4 |
| Earle's + Eagle's vitamins | 3.5 | 4.4 |
| " + Eagle's amino acids | 5.4 | 7.5 |
| " + glutamine 2 mM | 5.2 | 7.5 |
| " + .1% bovine serum albumin | 6.6 | 8.4 |
| " + "Tween 80" 20 γ/ml | 3.4 | 4.5 |
| " + penicillin 100 U/ml & streptomycin 0.2 mg /ml | 3.5 | 4.5 |

to have a similar stabilizing effect but so far none has been found more effective than bsa on a weight or molar basis. SFV prepared from infected chick embryos was found to have the same pattern of stability or loss of infectivity in 0.1 M phosphate in the presence or absence of bsa.

*SFV and WEE in tissue culture diluents.* When SFV or WEE were serially diluted in a series of tissue culture diluents, a high level of initial infectivity was obtained in all of them. However, after incubation at 37°C for 2 hours, infectivity persisted at a higher level in Medium 199 and Eagle's basal medium than in the 3 basal salt solutions which contain only inorganic salts in addition to glucose (Table I). Addition of some of the organic supplements in Eagle's basal medium to Earle's solution indicated that the persistence of a higher level of infectivity is associated with the presence of amino acids or glutamine rather than vitamins. Addition of "Tween 80," a nonionic surface active agent present in Medium 199, or antibiotics, did not appear to stabilize or enhance loss of infectivity in Earle's solution. Only in the presence of bsa was the virus titer after incubation at 37°C within the same range as preincubation titers.

*Discussion.* Despite differences in methodology the results of this study are consistent with most previous reports on both group A and B arboviruses. Howitt(2) found that at room temperature the infectivity of a filtered 20% suspension of WEE infected guinea pig brain was rapidly destroyed at pH 5.5 but was resistant at more alkaline pH. The infectivity of $10^{-2}$ dilutions of St. Louis encephalitis virus infected mouse brain persisted longer at 4°C at alkaline than at acid pH, as judged by further serial dilution and mouse inoculation(5). McLimans(6) studied the inactivation of $10^{-3}$ dilutions of WEE and EEE infected mouse brain by mechanical agitation and noted that above a critical pH of 6.4 both viruses were quite resistant to inactivation whereas at low pH they became readily susceptible.

Duffy and Stanley(7) reported that Japanese B encephalitis virus (JBE), as a $10^{-2}$ dilution of infected mouse brain in an acetate-glycine-phosphate buffer, was stable at pH 7 and 4°C but was rapidly inactivated at more acid pH. They also investigated a number of diluents for JBE virus and found that even at an optimum pH of 8, titers were lower when virus was diluted in non-protein containing inorganic salt solutions than in the same solutions with serum or skimmed milk added. This is consistent with our experience that great care must be taken to use chilled glassware and diluents in making dilutions in non-protein containing inorganic salts in order to get initial titers that are at all comparable to those obtained when using a protein-containing diluent.

A study of carefully purified EEE virus by Finkelstein *et al.*(8) showed a wide range of pH stability. They also noted a biphasic curve of pH stability for an unpurified chick embryo preparation of EEE virus, though not for the purified virus.

It has been known for many years that the loss of infectivity of yellow fever virus on dilution in isotonic saline may be prevented by addition of animal serum(1). Dick and Taylor(9) first demonstrated the advantages of bovine albumin in stabilizing yellow fever virus.

The mechanism by which serum albumin, even at very low concentration, prevents the loss of arbovirus infectivity is not known. Albumin accumulates at the surface of aqueous solutions and such surface activity is probably related in part to prevention of arbovirus inactivation by mechanical agitation(9). Surface activity may also be involved in thermal stabilization of SFV in the absence of mechanical agitation. Anionic surface active agents such as bile salts and sodium dodecyl sulfate inactivate both group A and group B arboviruses(10). This is not necessarily associated with surface activity *per se,* however, since both group A and B arboviruses are relatively stable in Eagle's basal medium in the presence of the nonionic detergent "Tween 80" at concentrations as high as 1% (Speir, unpublished). "Tween 80" present in Medium 199 in low concentration, preserves the complement fixing activity of purified poliovirus antigen by preventing adsorption onto glass surfaces(11). It does not,

however, prevent the loss of infectivity of SFV and WEE at 37°C in Earle's solution (Table I). Non-specific polymer interaction and specific protein-protein interactions are other mechanisms through which albumin could exert a stabilizing effect. Even highly purified preparations of albumin may contain significant amounts of other materials such as fatty acid(12), and such contaminants may be the stabilizing agent.

The mechanisms by which amino acids provide some measure of stability to SFV and WEE are also obscure. Cysteine has been reported to stabilize both EEE and WEE by acting as a reducing agent(13,14). Which amino acids are responsible for the increased resistance to inactivation indicated in Table I is not yet known; preliminary studies indicate that more than one may be effective. It is of interest that insects, a frequent vector of arboviruses, characteristically have high levels of free amino acid in their blood(15).

*Summary.* SFV retained a high level of infectivity from pH 6 to above pH 10 when serially diluted in 0.1 M phosphate buffer. After incubation of such serial dilutions at 37°C, infectivity persisted at $10^{-3}$ dilutions only above pH 6. The presence of 0.1% bovine serum albumin resulted in a marked increase in thermal stability at 37°C above pH 6 but not in more acid solutions. On dilution in several tissue culture media both SFV and WEE retained a higher level of infectivity in media containing a mixture of amino acids or glutamine than in media containing glucose and inorganic salts only.

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## Immunofluorescent Studies of Human Plasma Cells in γ and $\beta_2$A Myelomas. (28617)

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In human myeloma one can find a marked increase of γ globulins or $\beta_2$A globulin in the serum, and, in both cases, proliferation of plasma cells. We have tried to establish a correlation between myeloma protein production and cytologic abnormalities, using fluorescent specific antibodies.

*Material and methods.* Myeloma plasma cells were obtained by bone marrow aspiration. The aspirate (except for a part which was smeared and stained with May-Grünwald-Giemsa) was mixed with Hanks' solution containing heparin, centrifuged at 500 r.p.m. for 5 min., washed twice with Hanks' solution, smeared on coverslips, air dried and fixed by alcohol for 5 minutes at room temperature. In some experiments osmotic lysis of red cells was obtained, at least for most