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Prolonged Viability of Yellow Fever Virus in Serum Mixtures Containing Ammonium Sulfate.

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Because viruses tend to die out rather rapidly in liquid media if no growing cells are present, it is necessary to hasten all operations as much as possible while the virus under study is in this circumstance. When the experiments here described were performed little had been recorded about what constitutes the most favorable medium for yellow fever virus *in vitro*.[1-3] It was well known that simple saline solutions are rapidly lethal; and also that the presence of serum proteins exerts a profound preservative effect. However, under the best conditions the serum-saline mixtures are not especially effective. These considerations, together with the surmise that intracellular media are slightly acidic, led to trials with various inorganic salts which in the main tend to create a slightly acidic reaction. Following is a summary of what was found when ammonium sulfate was used.

Mouse brains infected with neurotropic yellow fever virus were ground and diluted with saline so that the final mixture contained a 10^{-2} dilution of mouse brain. Three mixtures were then prepared.

	I	II	III
Normal human serum	0.5 cc	0.5 cc	0.5 cc
10% ammonium sulfate	1.5 "	0.5 "	0.0 "
0.8% sodium chloride	2.5 "	3.5 "	4.0 "
Virus suspension 10^{-2}	0.5 "	0.5 "	0.5 "

Mixture No. I contains 3% ammonium sulfate; No. II contains 1%; and No. III contains none. The final strength of mouse-brain suspension is 10^{-3}. No. III represents the control, which is a mixture commonly employed in routine experiments with yellow fever virus. All 3 mixtures were incubated at 37° C continuously; material for test inoculations was removed daily under sterile precautions. Test animals were standard white mice; each received 0.03 cc of the inoculum intracerebrally in the manner which is standard for this work. The measure of viability of the virus was its capacity to cause illness and death in mice between the fourth and tenth days after intracerebral injection.

The control mixture (III) has lost much of its living virus after 48 hours of incubation, and practically all after 3 days. The mixture containing 3% ammonium sulfate in addition (I) shows a greater viability. The best exhibition is by the mixture containing one per cent ammonium sulfate; it retained much living virus through 6 days of continuous incubation, and appreciable amounts for 7 and 8 days.

Additional experiments established the fact that the optimal concentration of ammonium sulfate lies in the neighborhood of 1%. Another series of experiments showed that much benefit arises from a shift of the pH in the medium. When a buffered phosphate mixture of pH 6.3-6.5 is added instead of ammonium sulfate, a similar, though apparently not quite so great, prolongation of life of the virus is shown. The reactions of the ammonium sulfate-serum-virus mixtures soon after preparation were: (I) pH 6.2; (II) pH 6.3; (III) pH 7.4. After 5 days of incubation, the pH of each was 6.2, 6.5, and 7.7, respectively.

When one per cent ammonium sulfate mixtures containing virus and serum similar to the ones described are kept at room temperatures (22-25° C) and shielded from light, the capacity to kill mice in the usual fashion is maintained for as long as 20 days. Here again, the viability of virus in ammonium sulfate solutions is much longer than in normal serum dilutions alone.

[1] Lewis, P. A., *J. Exp. Med.*, 1930, **52**, 113.

[2] Milam, D. F., *Am. J. Trop. Med.*, 1939, **19**, 297.

[3] Frobisher, M., (a) *Am. J. Trop. Med.*, 1931, **11**, 127; (b) *Am. J. Hyg.*, 1930, **11**, 300; (c) *J. Exp. Med.*, 1931, **54**, 733.

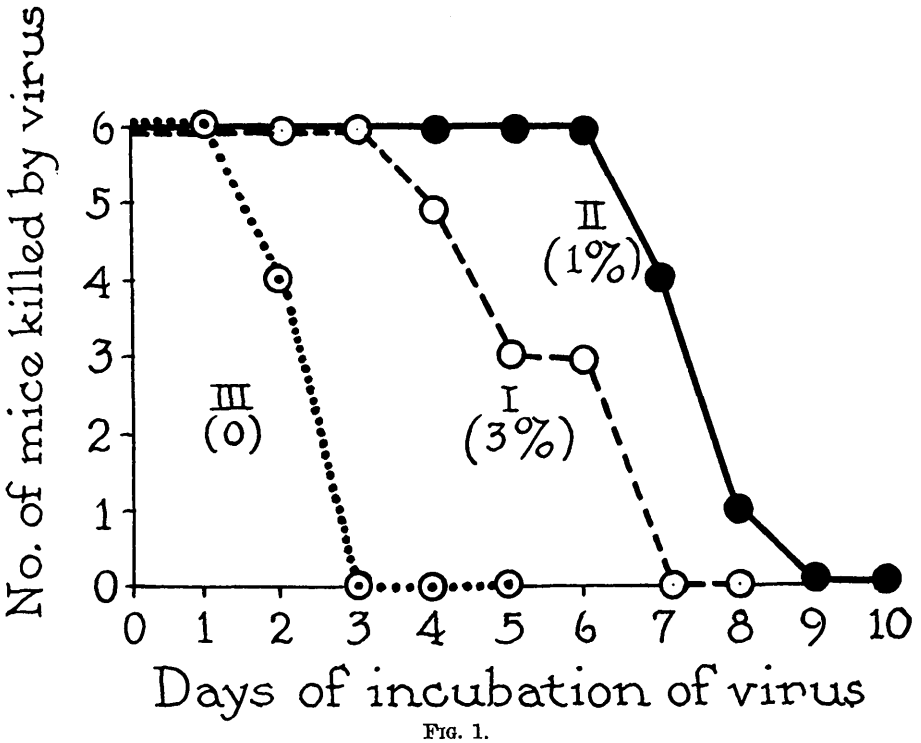

FIG. 1.
Experiment of September 17, 1941. Six mice were injected intracerebrally each day with each mixture. The graphs show the number of mice which died between the fourth and tenth days after injection.

Conclusions. (1) Neurotropic yellow fever virus in serum-saline dilution is protected by the addition of one per cent ammonium sulfate. (2) Much of the preservative effect is due to a shift of the reaction of the medium to the acid side. The optimal reaction appears to be near pH 6.3-6.5. (3) Addition of ammonium sulfate to 1% concentration seems to be the most convenient method of bringing about an optimal reaction.

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Estimation of the ED_{50} and Its Error by Means of Logarithmic-Probit Graph Paper.*

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With the increased emphasis on evaluation of all-or-none data there is need for a sound approximate procedure for arriving at a numerical expression of effect such as is represented by the ED_{50} (the dose affecting 50% of the group treated) and its standard error. The publication of Bliss[1] on the maximum likelihood solution of data based upon small numbers of subjects afforded a rigorous although somewhat tedious procedure which has been generally accepted. Using the latter for comparison, Irwin and Cheeseman[2] con-

[1] Bliss, C. I., *Quart. J. Pharm. Pharmacol.*, 1938, **11**, 192.

[2] Irwin, J. O., and Cheeseman, E. A., *Suppl. J. Roy. Stat. Soc.*, 1939, **6**, 174.