flavin and thiamine deficient animals were encountered by us in biotin deficient animals which had adequate supplies of these 2 members of the vitamin B group so that probably their experiments were complicated by multiple deficiencies.

Our data have also shown that vitamin C can be safely excluded from the hamster's ration since no growth differences were obtained when vitamin C was included or omitted from the diet.

Inositol or para-aminobenzoic acid or both may be needed in the ration of the hamster since deaths occurred when both these factors were excluded from the diet. However, experiments are now in progress to determine whether both or only one is needed.

Conclusions. A synthetic ration containing the 6 crystalline members of the B group which will support growth and reproduction in the rat was found wholly inadequate for the hamster. The addition of para-aminobenzoic acid and inositol increased the survival time of the hamster. A definite need for biotin was shown, the animals growing excellently and reproducing when it was included in the ration along with the 8 members of the B group including inositol and para-aminobenzoic acid. On the complete synthetic diet including biotin, inositol, and para-aminobenzoic acid, vitamin C and nicotinic acid are not required by the hamster.

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Effect of pH on Stability of Vesicular Stomatitis Virus.

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Most viruses are rapidly inactivated at high H ion concentrations, and therefore the report of Galloway and Elford[1] that the vesicular stomatitis virus (VSV) was still active after 24 hr at pH 4.51 and 0°C was of interest. Their results were based on qualitative tests, as a convenient method for titrating virus activity was not available at that time. Recently it has been found here that the 7-day-old chick embryo is quite susceptible to VSV inoculated on the chorioallantoic membrane and that it can be used to titrate the virus. Using the embryo to determine the amount of virus, the pH stability of VSV has been determined quantitatively.

The New Jersey strain of VSV originally obtained through the courtesy of Dr. P. K. Olitsky was employed. Seven-day-old chick embryos were inoculated on the membrane with an egg passage of the virus, and the infected embryos were harvested shortly after death. They were ground with 0.9% NaCl solution to make a 10% suspension by weight and the suspension was centrifuged in an angle centrifuge for 10 minutes at 4000 R.P.M. One cc of the supernatant was then added to 9 cc of buffer of the desired pH. The buffer used was the glycine acetate phosphate buffer described by Northrop.[2] It was chosen because it provides a constant ionic concentration in the medium, except for the H^+ and OH^- throughout the pH range studied. This buffer has been used before for a similar purpose.[3] It was found necessary to use the buffer undiluted, as it did not maintain its pH when the tissue-virus suspension was added if it had been diluted 1 part to 5 with saline (tested colorimetrically).

The buffer samples and the virus suspension were mixed in the cold and kept on ice in the refrigerator. Virus activity was then titrated

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1 Galloway, I. A., and Elford, W. J., *Brit. J. Exp. Path.*, 1935, **16**, 588.

2 Northrop, J. H., and DeKruif, P. H., *J. Gen. Physiol.*, 1922, **4**, 639.

3 Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, **72**, 49.

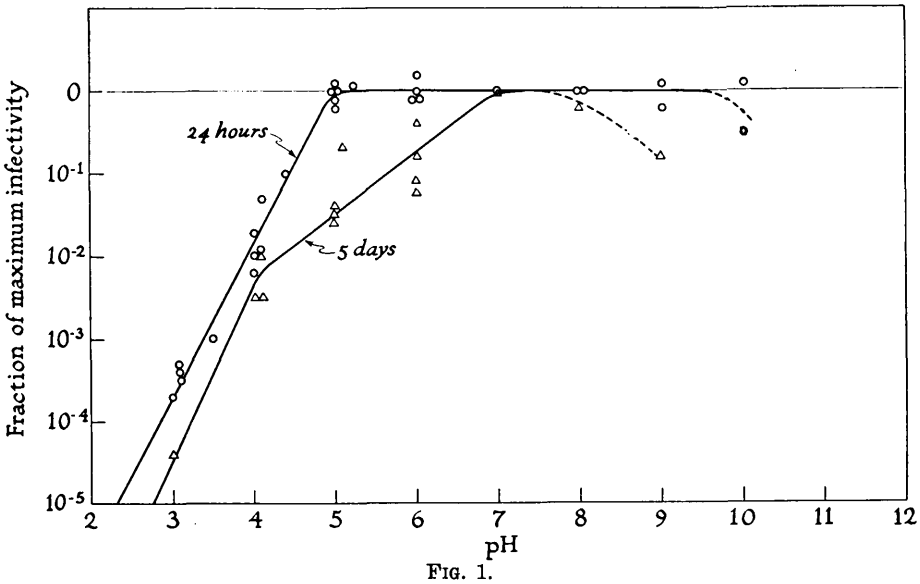

FIG. 1.

24 hours and 5 days later. Control samples with pH 7 were prepared and titrated simultaneously, and the inactivating effect of a given pH was the difference in titer found at the respective pH and pH 7.

For titrating virus activity serial tenfold dilutions of the virus were made in saline buffered to pH 7.4, and five 7-day-old embryos were inoculated on the chorionic membrane with each dilution. The eggs were incubated for 3 days at 35-36°C and the deaths noted. The point of 50% mortality could then be calculated.[4] Very few deaths occurred later than 48 hours after inoculation.

Fig. 1 shows the results of the titrations. After 24 hours below pH 5 the activity decreased rapidly. After 5 days 90% of the virus had been destroyed at pH 6. The irregular form of the 5-day curve is unexplained.

Summary. The activity of VSV suspensions was found unchanged between pH 4 and pH 9 after 24 hours at about 0°C. Increase in pH concentration beyond pH 7 brought about a progressive inactivation of the virus. After 5 days 90% inactivation had taken place at pH 6.

[4] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.