Onderstepoort Journal of Veterinary Research,
Volume 26, *Number* 2, *November*, 1953.
The Government Printer, Pretoria.

THE STABILITY OF NEUROTROPIC AFRICAN HORSE-SICKNESS VIRUS IN SOLUTIONS OF DIFFERENT CHEMICAL COMPOSITION.

A. POLSON, P. J. VAN ROOY, SYDNEY M. LAWRENCE and JOAN DENT,
Onderstepoort Laboratory.

In the preparation of virus vaccine emulsions for routine mass immunization in the field it is of the utmost importance that unaltered virus activity be maintained as long as possible. Apart from the influence of physical factors such as temperature and surface tension, the viability of the virus is dependent to a large extent on the chemical composition of the diluent in which it is suspended.

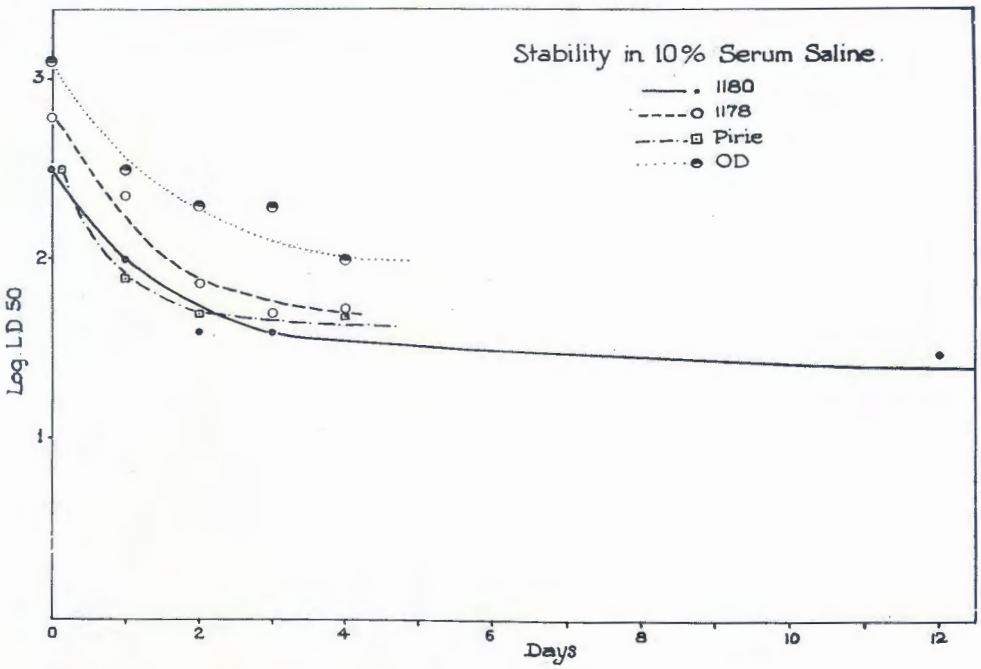

FIG. 1.—*Stability of four antigenically different strains of virus in* 10 *per cent serum saline at* 35° C.

At present the vaccine for horsesickness is issued from this laboratory in 10 per cent susceptible horse serum saline. There are, however, several disadvantages connected with the use of this medium as a diluent. These are the high cost of maintenance of the serum donors in an enzootic horsesickness area, the possibility of a horse harbouring an inapparent virus infection such as infectious anaemia

Received for publication on 28th June, 1952.—Editor.

and the inconsistency of the serum composition of horses which are subjected to frequent bleeding. The latter factor became apparent when sera of the donors were examined electrophoretically at the termination of the horsesickness vaccine production season after somewhat excessive bleeding over an extended period. Their sera showed very low albumin: globulin ratios when compared with those of normal horses (unpublished observations).

The present paper is a report of work done on the effect of different substances on the viability of horsesickness virus with the object of finding a substitute for serum-saline.

Method and Materials.

Mouse-adapted neurotropic virus of African horsesickness was used in the experiments.

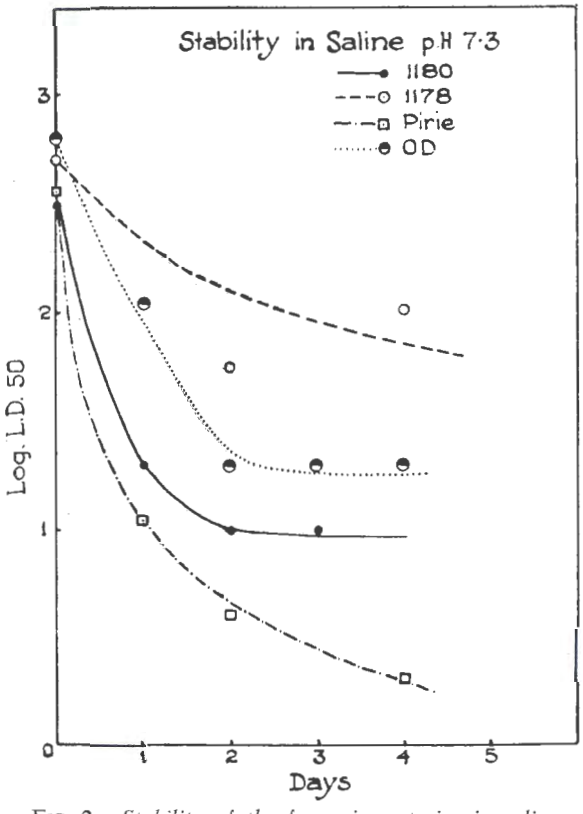

Fig. 2.—*Stability of the four virus strains in saline.*

The brains of four infected mice, killed *in extremis* were emulsified in 40 ml. saline and centrifuged for 20 minutes at 3,000 r.p.m. One ml. of the supernatant and 1 ml. 1 per cent Merthiolate* solution were added to 100 ml. of the test fluid and stored in an incubator at 35° C. The virus activity was determined at regular intervals by injecting serial two-fold dilutions intracerebrally into mice. Identical tests were made simultaneously on a 1 per cent solution of virus in 10 per cent serum saline and on a 1 per cent suspension in saline as standards for comparison.

* Merthiolate=Sodium ethyl mercuri thiosalicylate, Eli Lilly and Co.

Experimental.

A. In preliminary experiments the stability of four different strains of neurotropic horsesickness virus designated 1178, OD, 1180, and Pirie in 10 per cent horse serum saline and normal saline solution was investigated to determine which strain was most suitable for use in these experiments.

The results are graphically represented in figures 1 and 2.

Results.

From Figure 1 it is seen that the titres of the virus emulsions in serum saline showed in each case a sharp initial drop within 2 to 4 days to approximately one-tenth of the original value. In this particular experiment only the stablity of one strain, 1180, was followed beyond the 5th day. It was found that after the initial sudden drop the virus titre remained fairly constant up to the 12th day. This observation has been confirmed for the other strains in other experiments which it is not necessary to detail here.

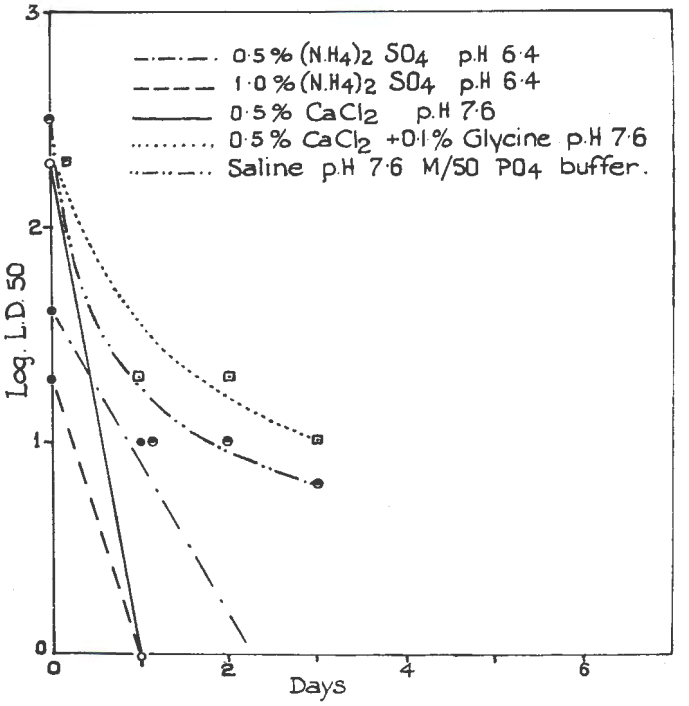

FIG. 3.—*Stability of Strain* 1180 *in Ammonium Sulphate, Calcium Chloride and Sodium Chloride solutions.*

From Figure 2 it appears that the decrease in viral activity in normal saline solution was most pronounced with strain Pirie, whereas the smallest decrease over the period was observed with 1178. Almost parallel curves for the viability of strain OD and 1180 were obtained in this experiment.

Variable results were obtained, however, when the stability of strain 1180 in NaCl solution of normal physiological strength was further examined. In some experiments virus stability in saline was almost identical with that in serum saline but more frequently it was found less stable.

A satisfactory explanation for this phenomenon cannot be given at this stage.

Strain 1180 was selected for use in the further experiments to be described since it appeared to be the least stable of the virus strains examined. In addition it has the advantage of a short incubation period in mice (three days) thus speeding up the work.

B. The stability of strain 1180 was then investigated in various solutions as follows:—

1. *Salt Solutions.*

In order to test the effect on viral stability of inorganic salts other than NaCl, one with a divalent cation, $CaCl_2$, and one with a divalent anion, $(NH_4)_2SO_4$, was selected. The results of these experiments are shown in Figure 3.

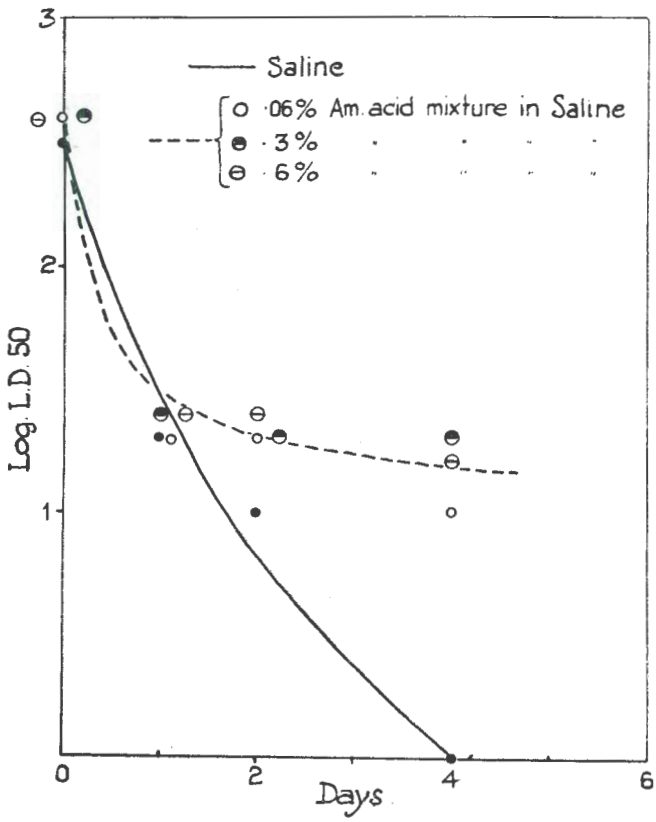

FIG. 4.—*Viability in amino acid mixtures in saline, and saline alone.*

Result.—The activity of the virus decreased rapidly in 0·5 and 1·0 per cent solutions of $(NH_4)_2SO_4$, pH = 6·4, and in a 0·5 per cent $CaCl_2$ solution, pH = 7·6. When 0·1 per cent glycine, pH = 7·6, was added to the $CaCl_2$ solution, active

virus, although in a considerably lower concentration could still be detected after three days incubation at 35° C. at which stage the virus titre was slightly higher than in 0·85 per cent sodium chloride in M/50 phosphate buffer pH 7·6.

2. *Solutions of Different Amino Acids.*

The stability of virus held in different concentrations of ten amino acids was then tested. 0·1 Per cent and 0·5 per cent solutions of glutamic acid (buffered at pH = 7·0), tyrosine, tryptophane, leucine, isoleucine, proline, histidine, valine, norvaline and serine were used.

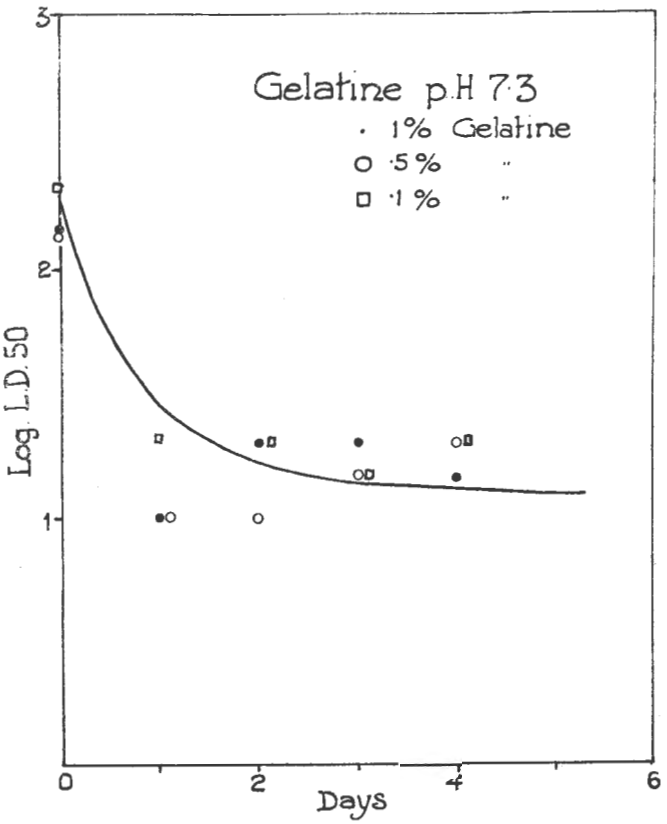

FIG. 5.—*Virus stability in* 0·1, 0·5 *and* 1·0 *per cent gelatine.*

With all these solutions a slight stabilizing effect, although much inferior to that of horse serum saline, was observed.

The effect of varying concentrations of mixtures of these amino acids was then compared with pure saline. The results are shown in figure 4.

Result.—No cumulative preservative effect of the amino acids could be detected and a level of constant virus activity again was reached after a sharp initial decrease. In saline alone the virus was inactivated by the fourth day.

3. *Gelatine.*

The viability in 0·1, 0·5 and 1·0 per cent gelatine was determined the result being given in figure 5.

Result.—It is seen that the virus is no more stable in the different concentrations of gelatine used than in amino acid mixtures. A corresponding level of active virus, equal to about 10 per cent of the original concentration, was again observed after the first day of incubation.

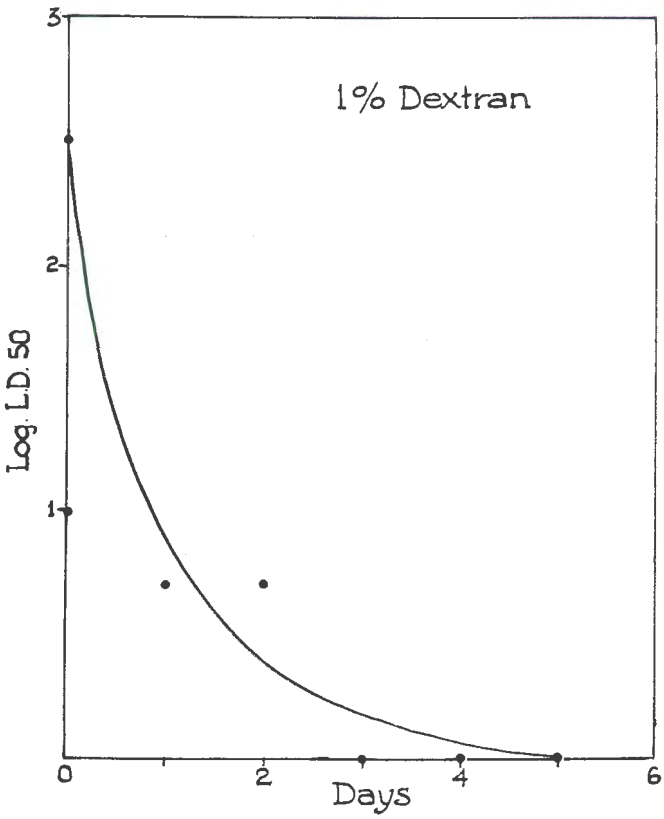

FIG. 6.—*Viability in 1 per cent Dextran.*

4. "*Dextran.*" *

Dextran was used as a 1 per cent solution, the stability curve being depicted in figure 6.

Result.—As shown in Fig. 6 only a trace of live virus was present in a 1 per cent. solution of "Dextran" after three days at 35° C. By the fifth day no active virus could be detected.

* Obtained from East Anglia Chemical Co. (Dextran) Ltd.

5. *Chick Embryo Extract.*

Normal eight-day old chick embryos were emulsified and 1·0 ml. of the supernatant fluid, obtained by centrifugation in an anglehead centrifuge at 3,000 r.p.m. for 20 minutes, was made up to 100 ml. with M/50 phosphate buffer. Viability tests in this solution gave results almost identical with those obtained in gelatine and mixtures of amino acids. The curve obtained is shown in Fig. 7.

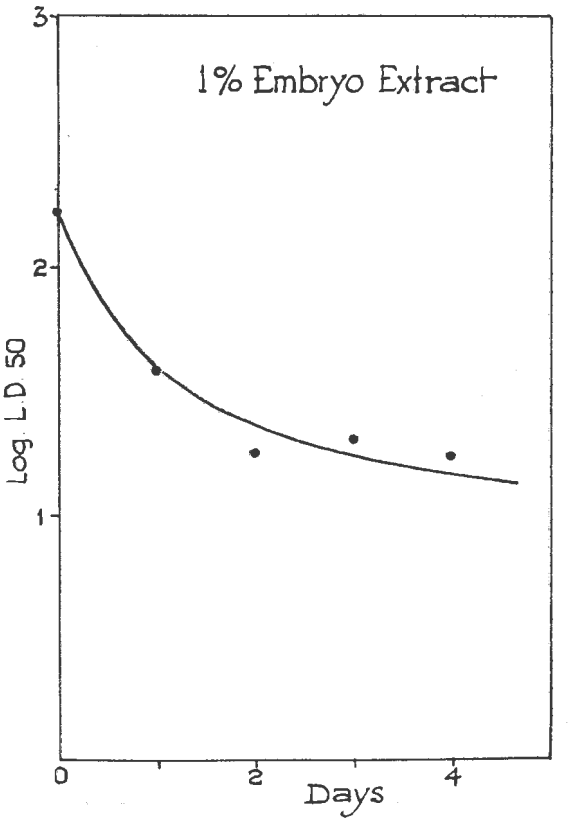

FIG. 7.—*Viability in 1 per cent chick embryo extract.*

6. *Egg White.*

Egg white in 0·1, 1·0, 5·0 and 10 per cent concentrations in M/50 phosphate buffer pH 7·6 was tested. The results are shown in figure 8.

Result.—It was found that the stability of the virus increased progressively with increase in concentration of egg white in a solution of M/50 phosphate buffer (pH = 7·2—7·4) over the range tested (from 0·1 per cent to 10 per cent egg white). It is seen from fig. 8 that the effect of a 5 per cent egg white solution is similar to that of 10 per cent horse serum saline, but practically no decrease in virus activity over a period of twelve days was observed when a 10 per cent solution of egg white was used.

Discussion.

Since strain 1180 is apparently the least stable of the vaccine strains examined, it was selected for use in a series of tests on the preservative effect of a number of diluents. Of these, solutions of calcium chloride, ammonium sulphate and "Dextran" had no value. In solutions of amino acids, gelatine, embryo extract, and normal horse serum a characteristic initial reduction in activity was observed after which the titre was maintained for relatively long periods at a level which varied with the diluent used. The stabilizing property of a 10 per cent solution of egg white in M/50 phosphate buffer, pH = 7·4, proved superior to that of any other solution tested; very little, if any, reduction in virus activity could be detected over periods of twelve days and longer at 35° C.

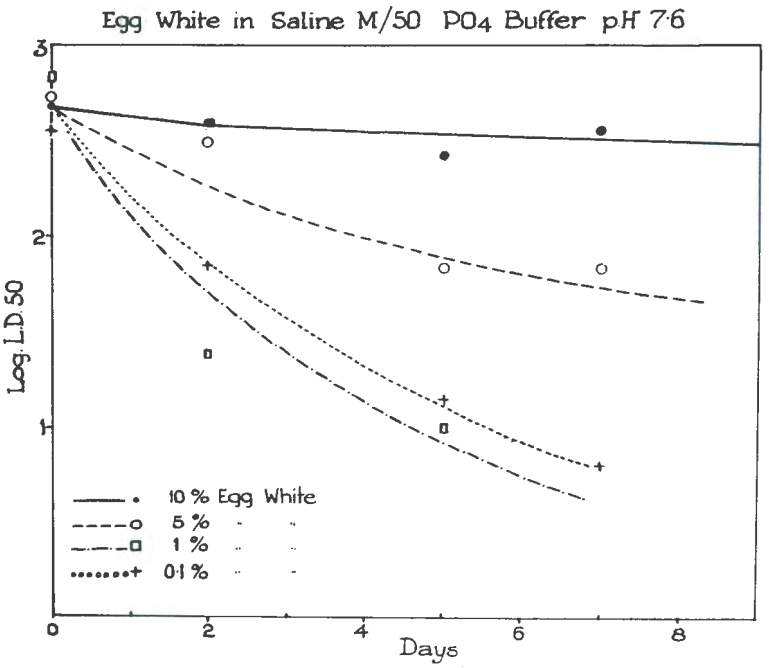

Fig. 8.—*Viability in* 0·1, 1·0, 5·0 *and* 10 *per cent egg white in M/50 phosphate buffer pH* 7·6.

From these results it appears that a 10 per cent solution of egg white, buffered at pH = 7·4, might replace the serum saline used at present as a verhicle for the virus in the vaccine. Naturally a number of other factors such as the development of sensitivity to egg white by animals treated remains to be investigated.

It is of interest to note that horsesickness virus showed different gradients of stability when stored in diluents of different composition. This effect is shown schematically in fig. 9.

The least stabilizing effect was shown by salt solutions and by "Dextran" (curve 1). In these media less than one per cent of the virus retained its activity after a few days incubation at 35° C. In solutions of animo acids, gelatine, embryo extract and 0·1 per cent and 1 per cent egg white (curve 2) there was an initial sharp drop in virus activity after which a level of relatively high stability

was reached. The virus which survived comprised about 10 per cent of the original. This gradient of stability is followed by that in 10 per cent horse serum saline and in 5 per cent egg white (curve 3). It is evident that about one-eighth of the original virus activity fell into this stability range. The highest level of stability was shown by the virus suspended in 10 per cent egg white solution. In this medium only a relatively small amount of the virus was unstable. No explanation for these observations can be offered at present. It was further observed that there was apparently some correlation between the stability of these strains in saline and their resistance to ultraviolet irradiation and it was also noticed that the stable strains were those that had previously been found to pass through Seitz E. K. filter-pads with difficulty (Polson 1941). These observations would indicate that the differences between strains are due to some surface phenomenon of the virus particles.

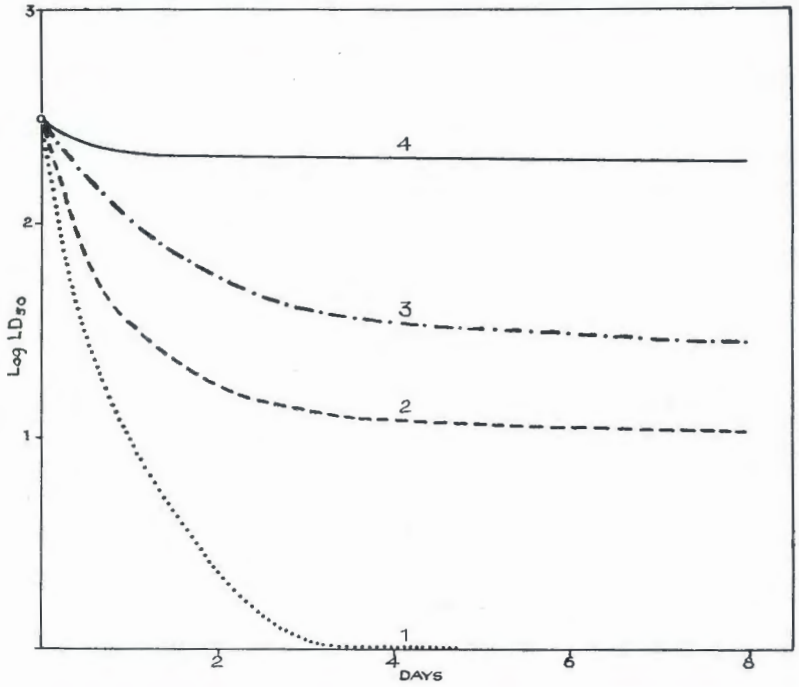

FIG. 9.—*Schematic representation of viability in various media.*

SUMMARY.

(1) The viability of neurotropic African horsesickness virus was tested in media containing various salts, different amino acids, gelatine, " Dextran ", chick embryo extract, serum saline and egg white. Of these, egg white was the medium of choice for retention of virus in a viable state.

(2) The virus of horsesickness showed distinct levels of stability in the various media. The highest level of stability was found in suspensions containing 10 per cent egg white, followed by 10 per cent serum saline and 5 per cent egg white solutions. At a lower level of stability were suspensions made up in animo acids, gelatine and embryo extract. A very low level of stability was shown by suspensions of the virus in salt solutions and in solutions of " Dextran ".

ACKNOWLEDGMENTS.

We wish to express our sincere gratitude to the Director of this Institute, Dr. R. A. Alexander, for his critical interest in this work.

REFERENCES.

POLSON, A., AND DENT, J. (1950). The rate of inactivation by ultra-violet irradiation as a means of distinguishing antigenically different strains of African horsesickness virus. *Brit. J. exp. Path.*, Vol. 31, p. 1.

POLSON, A. (1941). The particle size of African horsesickness virus as determined by ultra-filtration and ultra-centrifugation. *Onderstepoort J.*, Vol. 16, p. 33.