Wedensky inhibition, and thus giving rise to the first minimum. As the nerve impulses arrive with frequencies higher than 400/per second, about half of them would be extinguished in the absolute refractory period, leaving no inhibitory after-effect, and the other half coming later in the relative refractory phase would have greater chance of becoming effective. Hence the response-frequency curve rises again. But now as the stimulating frequency is increased beyond 500-600 per second, which is probably the maximum rhythm the nerve can follow, the propagated number of impulses per sec. actually become less and less with further increase of frequency of stimulation. When this number has dropped to 400-300 per second, Wedensky inhibition would again occur, hence a second minimum. With further drop in the impulse frequency the response frequency curve would naturally rise again, and would then fall a third time when impulse frequency becomes smaller than that necessary for calling forth the maximum contraction. The subsequent rise in the curve cannot be understood according to this scheme, but in this region of frequency of stimulation, summation of subliminal stimuli would probably occur and more muscle fibres become directly stimulated.

8363 P

Use of Saturated Sodium Chloride Solution for Preservation of Viruses.

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There are very few convenient methods for the preservation of viruses. The use of disinfectants is not permissible because of their deleterious effect upon viruses, while the commonly employed glycerol exerts only a feeble antiseptic action and, therefore, cannot be relied upon when freedom from bacterial contamination is important. The preservation of viruses through the process of drying *in vacuo* at low temperatures is an excellent method but it has the drawback of being somewhat cumbersome.

It has been shown by Ginsburg and Kalinin,[1] Sonnenschein,[2] and

[1] Ginsburg, S., and Kalinin, W., *Z. f. Immunität.*, 1929, **63**, 107.

[2] Sonnenschein, C., *Z. f. Immunität.*, 1930, **66**, 330.

others, that 10% solution of sodium chloride is effective in preserving complement for about 4 weeks. This action of sodium chloride upon complement, a substance of known instability, suggested to us that this salt may be a good agent for the preservation of viruses.

We found that saturation of guinea pig complement with sodium chloride preserves it for at least one month, and that various bacteria of the Salmonella group, pneumococcus, meningococcus, gonococcus, *Streptococcus hemolyticus* and *viridans, Staphylococcus aureus, B. influenzae* and *B. diphtheriae* are killed in from a few days' to one month's time when kept in saturated solution of sodium chloride.

For the present work we selected rinderpest and vaccine viruses and *B. dysenteriae* Shiga bacteriophage. The preservation of the first named virus is frequently a matter of great difficulty, especially in warm seasons, and, therefore, a method permitting its preservation even for a few weeks would obviously be of practical importance.

Rinderpest virus. A young Mongolian calf was artificially infected with rinderpest and killed at the height of febrile reaction. Pieces of spleen, measuring 5x3 cm., were placed in a glass stoppered bottle containing 100 cc. of saturated sodium chloride solution and kept in the ice chest. After 2 weeks a thick, coarse suspension of splenic tissue was prepared by grinding with saline and one cc. was injected subcutaneously into a normal young calf. Four weeks later 2 normal calves were similarly inoculated. All 3 animals developed typical rinderpest infection, with the usual incubation period of 3-4 days. Their blood was infectious for 3 normal calves. On necropsy these animals presented extensive ulceration of the abomasum. Clinically and pathologically the infection was associated with changes similar to those found in animals infected with fresh virus. This suggests that no (qualitative?) attenuation of the virus took place after 4 weeks of contact with saturated solution of sodium chloride.

Vaccine virus. Calf lymph was ground in a mortar with a small amount of 0.9% saline to form a thick paste. One-half was preserved by mixing with 4 times its weight of a solution containing 60 parts of glycerol and 40 parts of 1.8% phenol. The other was suspended in a similar quantity of distilled water saturated with sodium chloride. Each lot of vaccine was then subdivided, one portion being kept in the ice chest and the other at room temperature. Determination of their potency was performed at different time in-

tervals for 5 weeks. A rabbit was given intradermally 0.2 cc. of identical dilutions of 2 vaccines stored under similar conditions. During a storage of 5 weeks in ice chest, the titer of the vaccines gradually dropped from 1:10,240 to 1:1,280. No difference in the potency of glycerol and saturated salt solution vaccines was noted. Titration of vaccines kept at room temperature showed that during a period of 5 weeks the titre of glycerol vaccine decreased from 1:10,240 to 1:160 and that of saturated salt solution to 1:320. This slight difference was considered insignificant, although somewhat more intense reactions resulted from the vaccine preserved in saturated sodium chloride solution.

Dysentery Shiga bacteriophage, titre $1x10^{-10}$, was treated with an excess of sodium chloride and with an untreated control, distributed into tubes, sealed with paraffine and placed at 37°C. Titration of both lots of phage was performed from time to time for 3 months, by photometric measurements of turbidity[3] of young cultures of *B. dysenteriae* Shiga in broth to which phage was added. At the end of 3 months the potency of control phage had fallen from $1x10^{-10}$ to $1x10^{-5}$, while that of the salt-preserved phage dropped only to $1x10^{-8}$. This difference indicates that sodium chloride has a definite value in preserving the potency of phage. Its favorable effect has been further demonstrated by determination of the incubation time (time interval between the addition of phage and onset of bacterial lysis), using dilutions varying from $1x10^{-4}$ to $1x10^{-10}$. At the end of 3 months the incubation time of any dilution used in case of phage preserved with sodium chloride was 16 minutes shorter than that of the control lot. This indicates that the potency of salt-preserved phage was 400 times higher than that of the control phage.

8364 C

Use of Chinese Hamster for Testing the Virulence of C. Diphtheriae.

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The use of animals for the determination of virulence of *C. diphtheriae* is often necessary in the study of cultures obtained from patients or suspects. Frequently it is also desirable to use animals in

[3] Lin, F. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 488.