ence of larger amounts over a shorter period of time. Thus, it was found (Cori[5] *et al.*) that the continuous intravenous infusion of 0.00025 mm of epinephrine per minute produced maximal effect and that doubling or quadrupling of this amount produced no increased effect.

It is also well known (Cori[6]) that the intravenous injection of relatively large amounts of epinephrine is followed by hyperglycemia of short duration. By prolonging the absorption of epinephrine through the addition of zinc, epinephrine enters the circulation at a rate which is better for its economical utilization. This explains that epinephrine plus zinc not only has a longer lasting effect but that the maximum of this effect is also higher than when epinephrine is injected alone.

Summary. 1. The hyperglycemic effect of epinephrine in rabbits can be prolonged and increased by the admixture of Zn^{++} (in the form of $ZnCl_2$). 2. The prolonging effect of Zn^{++} depends on the concentration of the injected solution and the site of the injection. 3. Optimal results were obtained when the Zn^{++} concentration of the injected solution was 1.2% and the injections were made in the subcutis of the gluteal region.

5 Cori, C. F., Cori, G. T., and Buchwald, K. N., *Am. J. Physiol.*, 1930, **93**, 273.

6 Cori, C. F., *Physiol. Rev.*, 1931, **11**, 143.

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Relative Sensitivity of Different Phases of Growth Curve of *Bacterium salmonicida* to Alkaline Acriflavine.

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The action of acriflavine solutions on *Bacterium salmonicida* has been the object of several studies[1,2,3] because of its efficacy in the prophylactic disinfection of the exterior of trout eggs to prevent the spread of *Bacterium salmonicida* infection (trout furunculosis).

None of these reports has recorded the action of organisms tested in the various phases of the growth curve. Furthermore, a search of the literature has revealed no data on the relative sensitivity to lethal agents of the growth phases of an organism grown near its optimum temperature as compared to organisms grown near the minimum.

Using a modification[3] of the method of Ruehle and Brewer[4] for testing disinfectants, *Bact. salmonicida* cultures incubated for various periods of time and at 2 different temperatures (15°C and 10°) were exposed to the action of 500 parts per million (p.p.m.) of alkaline acriflavine.* The tests of the 15° cultures were run at pH 7.7, those grown at 10° at pH 8.0. The disinfection studies were run at 10°.

Table I summarizes the tests and Fig. 1 presents the death curve of the cultures.

The cultures grown at 15° showed little variation. There was a slight but definite increase in resistance to the dye as the culture aged. A peculiarity (autoagglutination) of the test organism prevented the procurement

1 Blake, Isobel, *Fisheries, Scotland, Salmon Fish*, 1930, No. 2, His Majesty's Stationery Office, Edinburgh, 1933.

2 Gee, Lynn L., and Sarles, W. B., *J. Bact.*, 1942, **44**, 111.

3 Smith, Winslow Whitney, PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 324.

4 Ruehle, G. L. A., and Brewer, C. M., U. S. Dept. Agri., Circ. 198, 20 pp., Washington, D.C., 1931.

* Abbott Laboratories Acriflavine lot No. 539126 used in all tests.

TABLE I.
Influence of Phase of Growth Curve on Sensitivity of *Bacterium salmonicida* to Acriflavine.

Days since subculture	½₄	1	2	3	4	5	6	7	8	9	10	12	14	16	18	21	24	27
Minutes to kill culture grown at 10°C	10	9.3	7.9	8	6.2	10	7.5	8.7	8.3	8.3	10	10	10	18	15	15	5	5
Minutes to kill culture grown at 15°C	5	7.5	5	5	7	5	5	8.2	5	8.2	6	7	7	8	7.5			

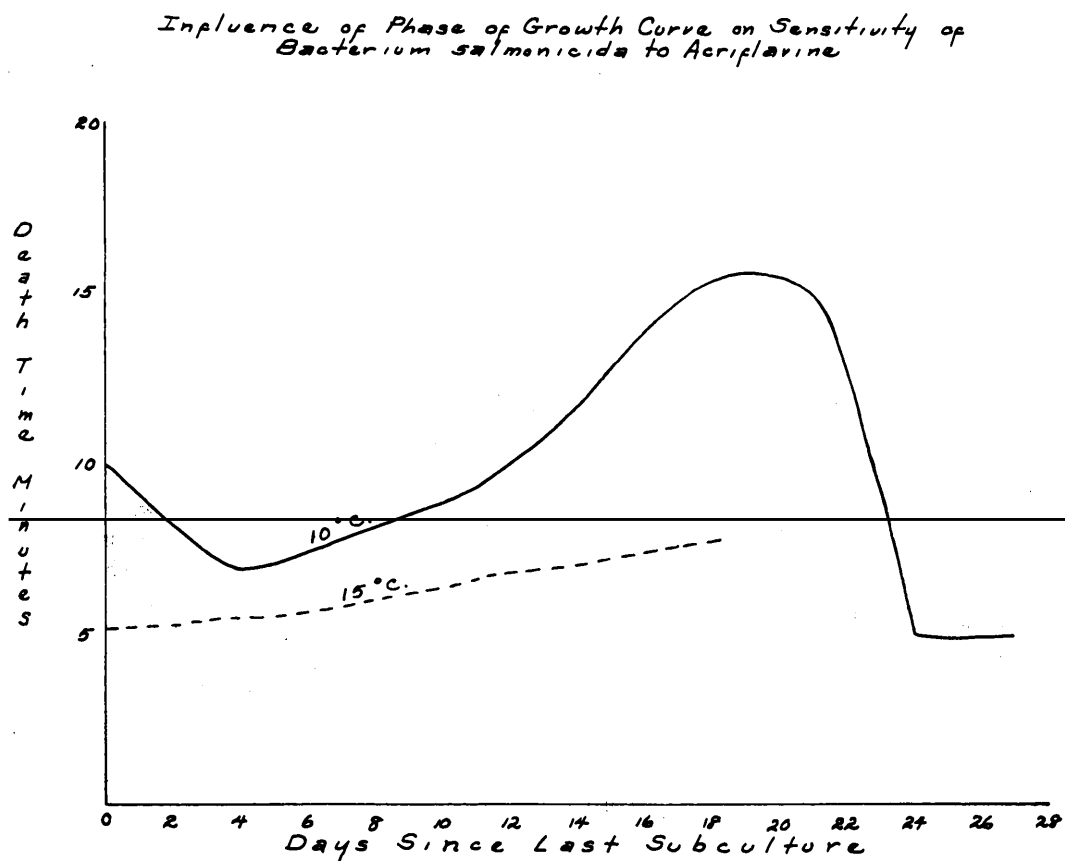

FIG. 1.

of bacterial counts. Therefore, it was not possible to compensate for the variations in load of organisms at each time interval. Had this been possible the death time of the youngest and oldest cultures would probably have been prolonged. The curve of death would have been U-shaped.

The 10° culture showed greater variation with age than the 15° culture. In what is assumed† to be the phase of exponential growth the organism was killed more rapidly than in earlier or later phases except that after 24 days all cultures were killed in 5 minutes. It is believed that had it been possible to correct for variations in load this curve would have shown a much deeper U

† The various phases of the growth curve cannot be positively identified. Thirty-eight different attempts to count the organisms in the various phases of the growth curve both by plate and direct count failed to yield conclusive data. There is, however, some indication that at 10° the lag phase ends in 5 hours, and the logarithmic growth phase in 48 hours. In 12 days the organisms were definitely in the phase of "logarithmic" death. The growth at 15° was faster.

shape than it does. Consequently, the 10° curve would have shown even greater relative variation when compared to the 15° culture. It is possible that the short death time of the 24- and 27-day cultures can be explained on the basis of reduced load of organisms tested.

It must be pointed out that since the 10° culture was studied at pH 8 and the 15° at pH 7.7 these 2 curves can be compared only as to shape, not as to magnitude.

The 2 incubation temperatures are believed to represent the optimum and the minimum because, of cultures grown at 5°, 7½°, 10°, 12½°, 15°, 17½°, and 20°, the 15° tubes clouded most rapidly. The 7½° showed no clouding.

These data indicate that for *Bact. salmonicida*—a Gram-negative psychrophile—organisms in the lag phase and the "logarithmic" death phase are more resistant to the action of 500 p.p.m. alkaline acriflavine solution at 10° than are bacteria in the phase of logarithmic growth. Organisms grown below the optimum temperature show a greater variation than those grown at the optimum.

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Production of Unidentified Vitamins by a Strain of *Mycobacterium tuberculosis* Grown on Synthetic Medium with p-Aminobenzoic Acid.*

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Mayer[1] reported the formation of a yellow pigment in cultures of a certain strain of Mycobacterium tuberculosis when grown in the presence of high concentrations of p-aminobenzoic acid (PABA). He suggested that the pigment might possibly be related to factors of the vitamin B complex. Since the solubility properties of the pigment were similar to those of vitamins B_{10} and B_{11}[2] which promote feathering and growth in chicks, and since PABA can partially replace these vitamins in chick diets,[3] it was decided to feed a culture filtrate of the organism grown in the presence of PABA to chicks on a diet deficient in the above vitamins.

The chick ration used (493K) contains, per 100 g: dextrin 61 g, alcohol extracted casein 18 g, gelatin 10 g, salts V[2] 6 g, soybean oil 5 g, l-cystine 0.3 g, choline 0.15 g, inositol 0.1 g, biotin 20γ, thiamin 0.3 mg, pyridoxine 0.4 mg, riboflavin 0.6 mg, pantothenic acid 2 mg, nicotinic acid 5 mg, 2-methyl-1, 4-naphthoquinone 50γ, and alpha-tocopherol 0.3 mg. Vitamins A (1200 I. U.) and D (120 I. U.) were fed by dropper weekly. Water and the experimental ration were given *ad libitum*. On this ration chicks grew slowly and feathered poorly, as shown in Table I. The addition of 2% liver fraction L (solubilized liver) or the Super filtrol eluate of liver fraction L[4] provided for rapid growth and good feathering.

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2 Briggs, G. M., Jr., Luckey, T. D., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1943, **148**, 163.

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4 Hutchings, B. L., Bohonos, N., and Peterson, W. H., *J. Biol. Chem.*, 1941, **141**, 521.