glucose consumption, which amounts during the last hours of life to 375 mg. per kilo per hour. The explanation of the increase must be sought in other directions, either in the incomplete breakdown of glucose to form other products than lactic acid, or in an inability of the animal to utilize fat as at first. There is no evidence of an increased metabolic rate during this period.

4598

The Relation of pH Value of Medium to Selective Bacteriostatic Action of Dyes.*

JOHN W. CHURCHMAN.

From the Laboratory of Experimental Therapeutics, Cornell Medical College.

Several years ago experiments were carried out in this laboratory to determine whether changes in pH value of the medium with which the experiments were conducted would alter the character of the selective bacteriostatic activity of gentian violet. In these experiments, the results of which were never published, a series of divided plates with the following pH values were planted with *B. coli, B. anthracis, Staphylococcus* and *B. prodigiosus:* 5.4, 6.4, 6.6, 7.4, 7.6, 7.8, 8.8 and 9.3. The upper halves of the plates contained gentian violet in a strength of 1 to 200,000. On all the plates from pH 6.4 to pH 9.3 selective action of the dye took place exactly as on media of pH 7.2; growth of the Gram positives was inhibited, growth of Gram negatives was unaffected. At pH 5.4 no growth of any organism occurred even on the plain agar. Means were not then at hand for buffering the media in the alkaline range beyond 9.3 but in plates made of media to which large amounts of alkali had been added, certainly sufficient to give a pH well beyond 10, no growth occurred even on the plain agar. The conclusion was reached that, within the range of growth, pH of media was not a factor in determining the character of selective action of gentian violet.

The recent publication of Dubos[1] in which the suggestion is made that some of the inhibitory dyes owe their power of inhibition to the fact that they poise the media at an oxidation potential outside the range in which the inhibited organisms can grow, made it seem wise to repeat our earlier experiments. Divided agar plates, the

* Work aided financially by the Chemical Foundation.

[1] Dubos, Réné, *J. Exp. Med.*, 1929, xlix, 575.

upper halves containing gentian violet 1 to 200,000, were, therefore, made of media at the following pH values: 3.6, 3.8, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, 6.0, 6.2, 8.5, 8.8, 9.3, 9.6, 10, 10.4, 11, 11.5, 12, 12.3, 12.7. To obtain the acid pH's HCl was added; to obtain the alkaline, sodium hydroxide. For the higher alkaline range M/10 CO_2 free sodium hydroxide and M/10 glycin solution were used as buffers.

In the acid range, at pH 5.2 selective action was as usual; at pH 5.0 *B. anthracis* failed to grow on the plain agar; at pH 4.6 there was no growth of any organism on either side of the plate. (See Fig. 1, a, b, c.) In the alkaline range selective action at pH 10 was as usual, beyond which point *Staphylococcus* and *B. anthracis* began to grow on the gentian violet side. (Fig. 1, d and e.) Since the dye had been obviously changed by the alkali, little significance could be attached to this fact. At 12.7 the selective action was as shown in Fig. 1, f. If plates were made of media to which still more alkali had been added (their pH could not be measured, but it was much higher than 12.7) no growth at all occurred on either side of the plate. (Fig. 1, g.)

The experiments were repeated in broth where the results were similar in principle, though the pH values at which growth failed to occur were slightly different.

These experiments seem to show that pH value of the media, if a factor at all, must be an insignificant factor in the selective

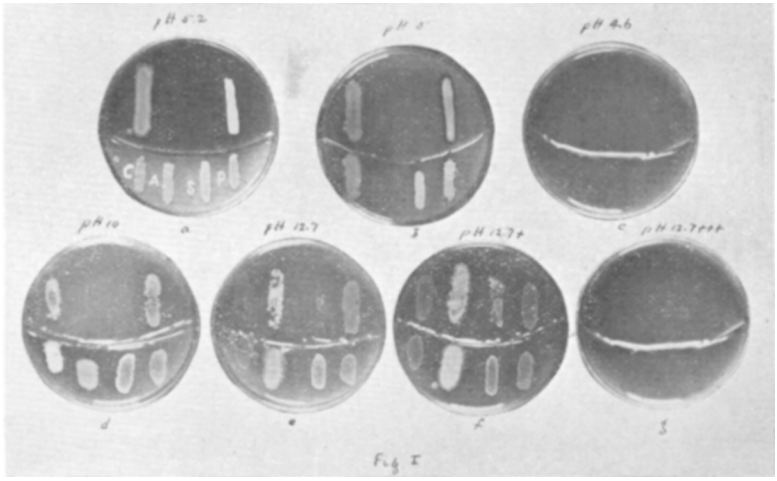
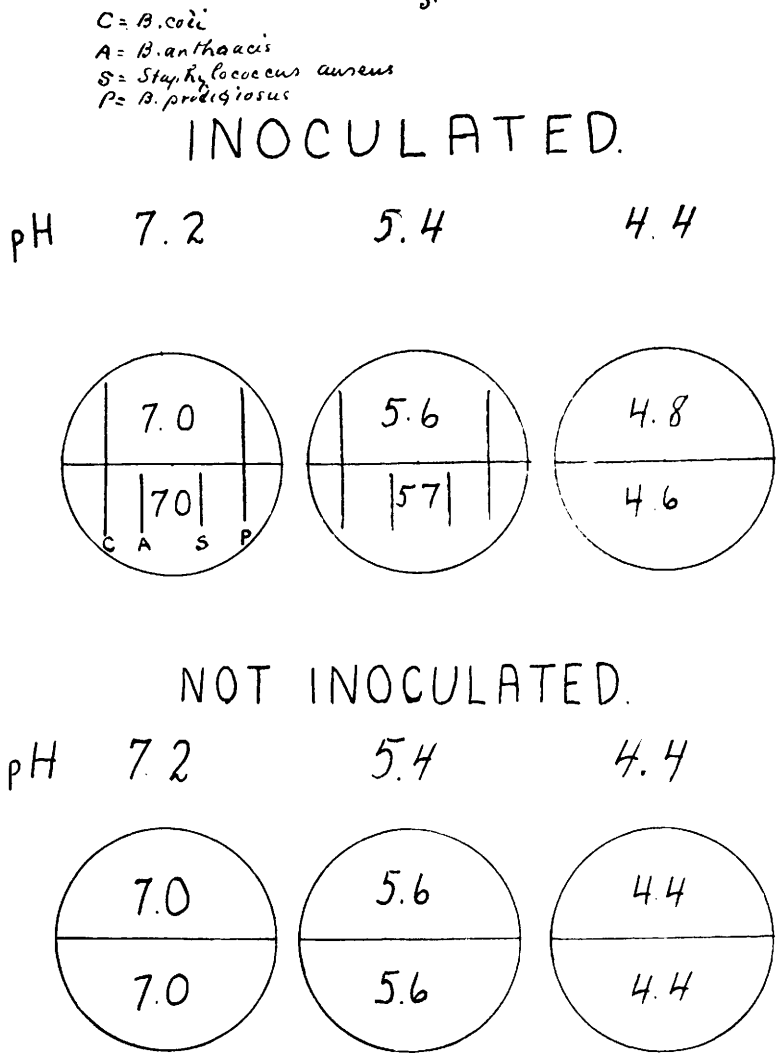


Fig. 1.

C = *B. coli.* A = *B. anthracis.* S = *Staphylococcus aureus.* P = *B. prodigiosus.*

bacteriostatic activity of gentian violet. It was thought that perhaps an error might have been made in drawing this conclusion, since there was a chance that the pH of the media used might have been changed by the bacterial growth or by the exposure of the plates in the incubator and, therefore, have been different at the moment the selective bacteriostasis took place from what it was when the plates were inoculated. Two series of 3 divided plates each were, therefore, made at pH's: 7.2, 5.4 and 4.4. Three of these were inoculated with *B. coli*, *B. anthracis*, *Staphylococcus* and *B.*

Fig. II



prodigiosus; while 3 others were put in the incubator without inoculation. After 24 hours the plates were removed. No growth at all had occurred on the 4.4 plate; on the other two, selective action had occurred as usual (see Fig. 2). The agars from each half of the plates were then carefully removed and their pH's taken. The results are recorded in Fig. 2. The pH of the dye containing agar was in every plate almost identical with that of the plain agar.

4599

The Use of Equations of the n-th Order to Describe the Action of Simple Haemolysins.

ERIC PONDER AND J. FRANKLIN YEAGER.

From Washington Square College, New York University.

In all recent work concerned with the fitting of formulae to curves obtained for the action of the simple haemolysins it has been assumed that the "fundamental reaction" between the cells and the lysin is one in which the latter combines with some component (probably protein) in the membrane of the former, thus forming a new compound as the result of the formation of which the integrity of the cell is destroyed. Thus, the quantity of the cell component, S, destroyed, is proportional to the quantity of lysin, x, used up in the system, and the velocity of the reaction is given by

$$(1) \quad dx/dt = k\,(c - x)$$

whence

$$(2) \quad t = \frac{1}{k} \log \frac{c}{c - x}$$

where c is the initial quantity of lysin (in milligrams), where t is the time required to produce lysis of an arbitrary number of red cells, and where S is large compared to c. Since it is assumed that the complete lysis of n cells corresponds to the utilization of a constant quantity of lysin, we obtain, by putting $x = \text{const.}$, and varying c in (2), a relation between the time for complete lysis of n cells and c, the initial concentration of lysin; when plotted, this relation gives the "time-dilution curve" for any particular lysin. If we are concerned with the number of cells, N, haemolysed from moment to moment by a particular concentration of lysin, from the beginning