4. In those experiments in which the urine volume has been followed, marked diuresis was observed, the rise in urine output apparently being directly related to the concentration of the blood and to the fall in blood sugar.

CONCLUSION

In insulin hypoglycemia a concentration of the blood is constantly found.

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Salt effects in bacterial growth: IV. The physical nature of bacterial growth in various concentrations of neutral salts.

By GEORGE E. HOLM and J. M. SHERMAN.

[*From the Research Laboratories of the Dairy Division, United States Department of Agriculture, Washington, D. C.*]

The physical nature of growth produced by various organisms has generally been attributed to the characteristics of the organisms themselves. Those organisms producing a pellicle upon the media in which they are growing were thought to do so because they were more strongly aerobic than those which did not form pellicles.

Of recent years, however, this view has been changed and it is known that organisms which grow in the surface of the media or settle to the bottom as they grow, do so, not alone because of the characteristic of the organism, but largely because of the physical properties of the media. It has been shown by Larson, Cantwell and Hartzell[1] that the nature of the growth of *B. subtilis, Mycobact. tuberculosis* and other organisms can be entirely changed by the addition of castor oil soap. In the case of *B. subtilis* 45 dynes prevents the formation of pellicles and growth takes place down in the body of the medium.

Neutral salts when added to the media in small quantities affect the physical nature of the growth of various organisms. *Bact. coli* when grown in medium of 1 per cent peptone broth

[1] Larson, W. P., Cantwell, W. F., and Hartzell, T. B., *J. Inf. Dis.*, 1919, xxv, 41.

whose H ion concentration has been adjusted between pH 5 and pH 8, shows great and varying degrees of turbidity, but the majority of the organisms settle out spontaneously. It has been noticed in our work, however, that when a small quantity of a neutral salt is added the majority of the bacteria are held in suspension and a very diffused growth is obtained.

Observations have been made upon media of varying H ion concentration in order to determine whether or not the action of the neutral salts is primary or secondary and to determine the relation of turbidity to the rate of growth in various concentrations of neutral salts.

EXPERIMENTAL

One per cent peptone was prepared and to various portions was added NaCl in quantities to make concs. of 0.05 M, 0.20 M, 0.30 M and 0.40 M. These media were then adjusted to H ion concs. of pH 5.3, 6.3, 7.0, 7.7 and 8.3, sterilized and inoculated with *Bact. coli.* Table I shows the results of these experiments.

TABLE I.

The Effect of H ion Concentration and Various Salt Concentrations of NaCl upon the Turbidity and Growth of *Bact. coli.*

pH of media	Order of turbidity of various NaCl concentrations					
5.3	0.05	0.10	C.	0.20	0.30	0.40
6.4	0.10	0.05	0.20	C.	0.30	0.40
7.1	0.10	0.05	C.	0.20	0.30	0.40
7.8	0.10	0.05	0.20	C.	0.30	0.40
8.3	0.20	0.10	0.05	C.	0.30	0.40

In the 1 per cent peptone controls the order of turbidity with regard to the pH of the media was as follows: 7.8, >7.1, >8.5, >6.4, >5.4. In our former experiments we have found that this is the order for various freshly prepared peptone media when placed in the order for optimum growth.

With the addition of increasing amounts of NaCl there seems to be a gradual shifting of the conditions for maximum turbidity in the pH range over which the experiments were carried out. The region of maximum turbidity shifts slightly toward the more alkaline side. The difference here is very small between the various H ion concentrations, as far as turbidity is concerned, and from the table given in the second paper of this series it was noted that the difference in the rate of growth in this region and

these concentrations of NaCl is very small. In other words, the conditions for maximum turbidity or diffused growth seem to parallel very closely the optimum conditions for rate of growth.[2]

A series of sodium salts with various anions were compared in order to obtain their relative effects upon the nature of the growth. NaI, $NaNO_3$, Na_2SO_4, and Na acetate were tried in concentrations ranging from 0.05 M to 0.40 M.

TABLE II.

The Effect of Various Concentration of Different Sodium Salts upon the Physical Nature of Growth of *Bact. coli* at pH 7.6.

Salts used.	Order of turbidity of the various salt concentrations.					
$NaNO_3$	0.10	0.20	0.30	0.40	0.05	C.
NaI	0.10	0.20	0.30	0.05	C.	
Na_2SO_4	0.10	0.05	0.20	C.	0.30	0.40
Na acetate	0.10	0.20	0.30	0.40	0.05	C.

Sodium iodide and sodium nitrate both give growths of a more highly dispersed character than the growth in the controls, even in concentrations of 0.30 and 0.40 M. Sodium sulfate, on the other hand, shows more dispersed growth than the control only in concentrations of 0.05, 0.10, and 0.20 M. On the other hand, 0.30 and 0.40 M sodium sulfate peptone solution give granular growths, showing the action of the SO_4 ion. Na acetate gives approximately the same results as NaCl.

When 0.20 M concentrations of these salts are compared with the same concentration of NaCl the order of the turbidities was found to be as follows: $NaNO_3$, >NaI, >NaCl, >control. This order again parallels very closely, as far as can be judged under the conditions used, the order found for the rate of growth. Upon allowing these cultures to stand for a time after they have been incubated for from 6 to 8 hours it was noted that the tendency for settling out was but slight in the media which showed maximum turbidity. From analogy with the effect of neutral salts upon colloidal properties in general, the dispersed growth which has been explained might be due to the initial lowering of the surface tension by small quantities of a neutral salt added to 1 per cent peptone. The magnitude of change of surface tension of 1 per cent peptone with added amounts of salts was measured.

[2] Holm, G. E., and Sherman, J. M., *J. Bact.*, 1921, vii, 511; Sherman, J. M., and Holm, G. E., *J. Bact.*, 1922, vii, 465.

TABLE III.

Changes in the Surface Tension of Peptone upon Addition of Neutral Salts.

Media	pH	Surface tension (dynes/cm^2).
1 per cent peptone	5.0	58.03
" — .20 M NaCl	5.0	57.95
" — .10 M Na_2SO_4	5.0	57.68
" — .20 M Na_2SO_4	5.0	57.30

Table III indicates that there is but a slight decrease in the surface tension of 1 per cent peptone when small amounts of NaCl or Na_2SO_4 are added. The changes are so small, however, that it would be impossible to attribute the cause of dispersed growth to the changes in the surface tension. Furthermore, it was found that 0.20 M Na_2SO_4—1 per cent peptone media. It is hardly probable, therefore, that the phenomena of dispersed growth obtained when small amounts of neutral salts are added is due entirely to changes in surface tension.

Hofmeister[3], and Fischer[4] showed that small amounts of neutral salts increase the swelling of proteins. The Cl, NO_3, and I ions were found to be the most efficient in this respect. Hofmeister and Bechhold and Ziegler[5] showed that diffusion through membranes may be increased by the presence of chlorides, nitrates, iodides, etc. The permeability of plant plasma for neutral salts has been studied by Kahho,[6] who found that iodides, bromides, chlorides, etc., favored permeability more strongly than the sulfate and citrate. He also noted that all ions which increased permeability also increased susceptibility to toxic action. It has been shown by Lemon[7] that bacteria are less resistant to the action of phenol in the presence of small neutral salt concentrates than in the ordinary media in which they are in suspension. The phenomenon is in all probability due to the increased permeability of the bacterial cell.

Entirely in accord with these findings we may conclude that in the presence of small amounts of neutral salts the permeability of the cells is greater than the permeability of the same bacterial cells in the plain 1 per cent peptone solution. This may mean

[3] Hofmeister, F., *Colloids in Biology and Medicine*, p. 346.

[4] Fischer, M. H., *Colloids in Biology and Medicine*, p. 80.

[5] Bechhold, H., and Zeigler, J., *Colloids in Biology and Medicine*. pp. 55, 346.

[6] Kahho, H., *Biochem. Z.*, 1921, cxxiii, 284.

[7] Lemon, J. S., *J. Phys. Chem.*, 1920, xxiv, 570.

more highly hydrated and larger cells, which would tend toward greater stability of the suspension. This is in accord with the phenomena observed by Henrici,[8] through microscopic measurements of the size of *B. megatherium* during the lag period and during the period of most rapid growth. It was found that during the period of most rapid growth the organisms are larger in size than during the lag period or during the period subsequent to the period of rapid growth.

We have shown in previous work that small amounts of neutral salts increase the rate of growth in 1 per cent peptone and we believe that this increase in the rate of growth is closely related to the permeability of the bacterial cell. Largely because of the observation that there is a remarkably close correspondence between optimum salt concentration for maximum turbidity or diffused growth, and the optimum salt concentration for the maximum rate of growth we are inclined to believe the effect of the salts, whatever it may be, is in this case an effect upon the organisms themselves rather than an effect upon the physical property of the media.

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On the factors influencing the appearance of placques of bacterial lysis.

By J. BRONFENBRENNER and CHARLES KORB.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

If the activity of "bacteriophage" is studied upon agar cultures spots or so-called placques of lysis are seen to appear, instead of a homogeneous growth. As a rule, these clear spots stand out quite distinctly against the background of agar, but occasionally one observes among them a certain number of spots which may be considerably less distinct than others. The variations among the spots are even more marked as regards

[8] Henrici, A. T., PROC. EXP. BIOL. AND MED., 1924, xxi, 215.