CHANGES IN THE STABILITY AND POTENTIAL OF CELL SUSPENSIONS.

I. The Stability and Potential of Bacterium coli.

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(Received for publication, June 4, 1923.)

In a previous investigation (Eggerth and Bellows, 1921-22) a strain of *Bacterium coli* was used that agglutinated in acid salt mixtures at pH 3.3, and remained unagglutinated at pH 3.0 and 3.5. It was observed that when agglutination at pH 3.3 was completed (after 1 or 2 hours at 40°C.), that if the tube was thoroughly shaken, the agglutinated bacteria went into even suspension and failed to flocculate again even when kept for days at that pH. Frequently microscopic examination showed that agglutination in small microscopic flocculi persisted, but if the treatment with acid was continued for another hour or two, vigorous agitation produced reemulsification so complete that not a trace of microscopic flocculation was demonstrable. Furthermore, such an acid-treated suspension, when sedimented on the centrifuge and resuspended in a series of buffer solutions, failed to agglutinate at any reaction between pH 7.0 and 2.4.

The following experiment was undertaken. An 18 hour agar culture of this strain of *Bacterium coli* (which will henceforth be referred to as the Goldberg strain) was washed four times in distilled water. Suspensions were made in lactic acid-sodium lactate buffers at pH 2.4, 2.7, 3.3, 3.8, and 4.4, and in acetic acid-sodium acetate buffers at pH 5.0 and 5.6. These were incubated at 35°C. for 3 hours. The buffer solutions were made up according to Beniasch (1912), except that the final salt concentration was made 0.01 N. After incubation, the suspensions were sedimented on the centrifuge, taken up in distilled water, and distributed in the lactate series of buffers.

As it was found that the density of the bacterial suspension made some difference in the results, the *coli* suspensions were compared with a standard suspension of staphylococci, and the turbidity in the agglutination tubes was made equivalent to that of a staphylococcus suspension of 2 billion per cc.

The results of this experiment are shown in Table I. Treatment at pH 3.3 and reactions acid to this prevented agglutination unless

TABLE I.

pH at which incubation for 3 hrs. was maintained.	Flocculation. Lactate buffers. pH								
	4.7	4.4	4.1	3.8	3.5	3.3	3.0	2.7	2.4
2.4	−	−	−	−	−	−	−	−	−
2.7	−	−	−	−	−	−	−	−	−
3.3	−	−	−	−	−	−	−	−	−
3.8	−	−	−	−	−	+	+	+	+
4.4	−	−	−	−	−	+	+	−	−
5.0	−	−	−	−	−	+	−	−	−
5.6	−	−	−	−	−	+	−	−	−
3.3*	−	+	−	−	−	−	−	−	−
3.3†	−	+	+	+	+	+	−	−	−
3.3‡	−	−	+	+	+	+	−	−	−
Control	−	−	−	−	−	+	−	−	−

The final concentration of sodium lactate was 0.01 N. Temperatures = 35°C.
* Washed three times in distilled water after neutralization of sediment.
† " " " " 1 per cent NaCl.
‡ " " " " 2 " " $NaHCO_3$.

the acid-treated bacteria were washed. At pH 3.8 and 4.4 the effect was to widen the zone of flocculation. Treatment with buffers at pH 5.0 and 5.6 gave agglutination as in the control. Attempts to reverse the effect of acid treatment of these bacteria by neutralizing the sediment and washing with distilled water, 1 per cent NaCl, or 2 per cent $NaHCO_3$, changed the stability of the bacteria once more; flocculation reappeared, but the zone of flocculation was widely displaced toward the alkaline side.

Experiments were then undertaken to determine if any changes in the potential difference between the bacteria and the fluid accompanied these alterations in stability. For this purpose, a microcataphoresis apparatus made according to Northrop (1921–22) was used. The depth of the cell was 1 mm., and the fall in potential of the current was 5 volts per cm. The principles of this apparatus are fully explained by Northrop. In one way only was his procedure departed from: instead of making measurements at four depths in the upper half of the cell only, measurements were made in the middle of each sixth of the entire cell depth. Theoretically, the upper and lower halves of the Northrop cell are symmetrical; when using a new, clean cell, this is actually the case. But after a little use, the glass walls of the cell become coated with bacteria, as was described by Northrop (1921-22), and the migration of water along the cell wall is changed. As the bottom of the cell acquires a thicker coating of cells in a shorter time, the two halves are no longer symmetrical. In most of the observations for this paper, the rate of movement was distinctly slower in the lower half of the cell than in the upper, although this varied with the suspension used. In cataphoresing bacteria, the error produced in making measurements in the upper half of the cell only is not large; but when heavier cells, such as erythrocytes, are used, this error becomes very great.

The rate of movement of the bacteria is given in the figures as micra per second with a calculated fall in potential of 1 volt per cm. The potential in millivolts between the surface of the bacteria and the fluid is given as calculated from the observed velocity by means of the Helmholtz-Lamb equation, as discussed by Northrop and Cullen (1921–22). In these cataphoresis experiments, the bacteria were always washed four times in distilled water and cataphoresed on the same day. Unless otherwise specified, the buffer solutions were lactic acid-sodium lactate mixtures containing 0.01 N salt.

Different suspensions of *Bacterium coli* do not always have the same charge at a given pH. Thus, at pH 4.7, the cataphoretic potential of the Goldberg strain varies between 34 and 42 millivolts in the buffer used. Northrop and De Kruif (1921–22, *a*) noted similar variations in their suspensions of *Bacterium typhosum*, which they ascribed to differences in the age and condition of the suspen-

sion. No such variations, however, occurred when observations were made on the same preparation of *Bacterium coli* at the beginning and end of an experiment.

Fig. 1 shows the changes in charge of the Goldberg strain when incubated at 35°C. at different reactions. At pH 4.1 and 5.3 there was no appreciable change within 7 hours. At pH 3.8, the curve remained flat for 2 hours after which the negative charge diminished until at the end of 6 hours it was one-third of its original value. At more acid reactions, the velocity of this change in charge is greatly accelerated; at pH 2.4, the charge fell to zero within 45 minutes.

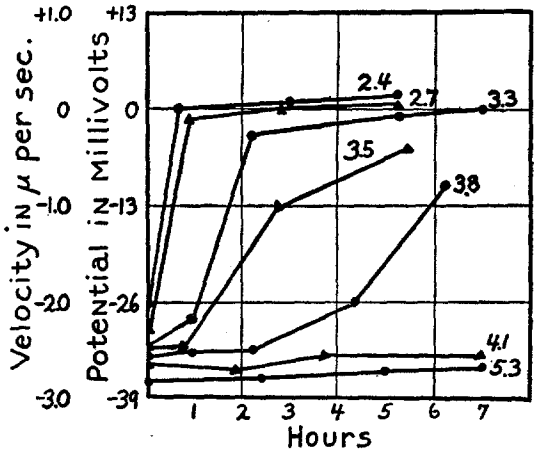

FIG. 1. Effect on the potential of *Bacterium coli* suspensions when incubated at 35°C. in 0.01 N lactate solutions at the pH indicated on the figure.

Similar experiments conducted with acetate and phthalate buffers, and with mixtures of HCl and KCl, gave the same results.

At intervals during this experiment, the *coli* suspensions were plated out on agar media to test for viability. Though microscopic agglutination interferes with the strict accuracy of the results, it seems that there is little cell death during the period of the flat portions of the curves, but that the bacteria are dying rapidly as the curves rise steeply. It is probable that cell death and change in charge are both results of profound physical and chemical alterations of the cell.

In Fig. 2, the charge of acid-treated bacteria is compared with that of normal controls at different reactions. Curve *A* gives the charge of acid-treated bacteria which were centrifugated and resuspended in the different buffer solutions without further washing. Curve *B* shows the same acid-treated bacteria, neutralized, incubated for 6 hours in a 0.01 N phosphate solution at pH 7.6, and then washed in distilled water. Acid treatment diminished the negative charge at all reactions between pH 6.4 and 2.4, especially at the acid end of this series. The attempt to reverse the effect of acid treatment (Curve *B*) produced only inconsiderable changes, most noteworthy being the increase of the positive charge at pH 2.7 and 2.4, which occurred very constantly in different experiments. When the acid-treated bacteria were washed three times in 1 per cent NaCl or 2 per cent $NaHCO_3$, curves were obtained that differed very little from Curve *B*, and are therefore omitted.

The exact position and inclination of Curves *A* and *B* of Fig. 2 varied somewhat in the different experiments as the initial charge and the duration of treatment varied. Prolonged acid treatment caused Curve *A* to rise much more steeply, and *vice versa*. Curve *B* also varied with the length of acid treatment and the length of the after treatment, lying sometimes to the left, sometimes to the right of Curve *A*, though never far from it.

Figs. 1 and 2 have to deal with the Goldberg strain of *Bacterium coli*. Ten other strains of this organism were isolated and tested for similar behavior. By referring to Table II it will be seen that each of the other ten strains has its original negative charge diminished by acid treatment, some even acquiring a considerable positive charge. The phenomenon here studied is not, therefore, a peculiarity of this one strain. No other species of bacteria have as yet been studied in this manner, but experiments with human and sheep erythrocytes show that similar changes of potential take place in red cells. These will be reported in a later paper.

When fresh washed bacteria (the Goldberg strain) were added to a suspension that had been incubated for some time at pH 3.3, it was found that all of the bacteria in the field, both new and old, moved with the same velocity (usually zero) when cataphoresed. When a suspension that had been treated at this reaction until the

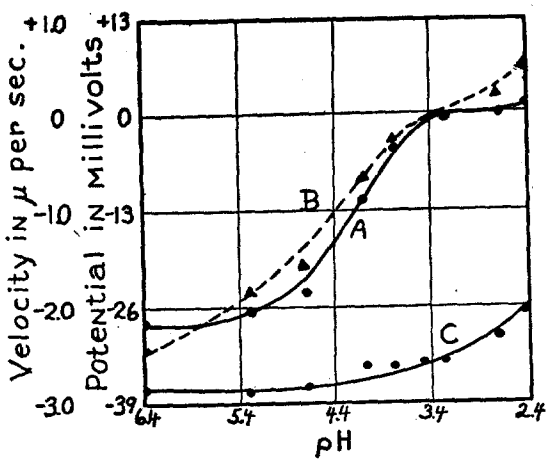

FIG. 2. The potential of *Bacterium coli* suspensions in 0.01 N lactate solutions; (*A*) after being incubated for 3 hours at 35°C. at pH 3.3, (*B*) after being thus treated at pH 3.3, then incubated for 6 hours at pH 7.6 and washed once in distilled water, and (*C*) the potential of washed but untreated *Bacterium coli*.

TABLE II.

Strain of *Bacterium coli*.	Fresh bacteria in fresh buffer. (1)	Fresh bacteria in acid, supernatant. (2)	Acid-treated bacteria in fresh buffer. (3)
	millivolts	*millivolts*	*millivolts*
Resa	−10.5	0	+3.0
Tucker	−16.0	0	+11.0
Siegel	−15.0	−7.5	+5.5
Wilkins	−22.0	−9.0	−5.5
Peck	−1.5	0	0
Matthews	−23.5	−5.5	−4.5
Penny	−16.0	+8.5	+7.5
Gert	−21.5	−20.0	−10.0
Mouse	−5.5	0	0
Cameron	−21.0	0	−2.5
Goldberg	−34.0	0	0

Potentials in 0.01 N lactate buffer at pH 3.0.

potential of the cells was zero, was centrifugated, and fresh cells added to the clear supernatant fluid, cataphoresis showed that the charge of the new cells was now zero, whereas fresh cells in fresh buffer at this pH usually have a charge of about 35 millivolts. Not only are the cells altered by acid treatment, but the fluid in which they are suspended acquires new properties.

In order to study this phenomenon in greater detail, an "acid extract" was prepared by adding a HCl-KCl mixture at pH 1.8 to a heavy suspension of *Bacterium coli* Goldberg, making a final salt concentration of 0.01 N. Enough alkali was liberated by the bacteria to depress the H ion concentration to a pH of 3.0 (colorimetric). After 4 hours at 35°C., the suspension was sedimented on the centrifuge and the clear supernatant fluid brought to pH 7.0. This neutralized "acid extract" gave a distinct turbidity with alcohol and foamed strongly on shaking; a micro Kjeldahl determination gave a total N of 10 mg. per 100 cc. Assuming this to be all protein nitrogen, the protein concentration was 1 part in 1,600.

The neutralized "acid extract" prepared in this way was now added in varying quantities to fresh cells in fresh buffer solutions, determinations being made immediately. Results are given in Fig. 3, which shows that even small amounts of the "acid extract" depress the charge to some extent, while larger amounts depress it a great deal especially in the more acid reactions.

When Fig. 3 is compared with Figs. 1, 2, and 3 of the paper of Northrop and De Kruif (1921–22, *b*), it will be seen that this "acid extract" behaves like any other protein in diminishing the negative charge on the bacteria. The "acid extract" is liberated from the bacteria, and then, in part, combines with their surfaces. It is possible that this protein is similar to that extracted by Arkwright (1914) from *Bacterium typhosum*, though the "acid extract" here described differs from Arkwright's extract in two important ways: it is not obtained at neutral reactions, and it does not flocculate at any pH. Undoubtedly Arkwright's extract contained a great deal of peptone and other substances from the media on which the germs were grown.

The other ten strains of *Bacterium coli* were now tested to see if they too produced an active "acid extract." The washed bacteria were treated at pH 3.0 for 10 hours at 35°C. (0.01 N lactate buffer)

and sedimented on the centrifuge. Fresh washed bacteria of the homologous strain were now emulsified in the clear supernatant fluids and cataphoresed (Column 2, Table 2). The sediments were suspended in fresh buffer at pH 3.0 and also cataphoresed (Column 3). In Column 1 are found the potentials of fresh washed bacteria at this pH. Although the results obtained are very diverse, they agree essentially with the facts ascertained by the more detailed study of the Goldberg strain.

We are now in a position to make a partial interpretation of the agglutination experiment shown in Table I. The bacteria treated at pH 3.3 and more acid reactions have become covered with a film of

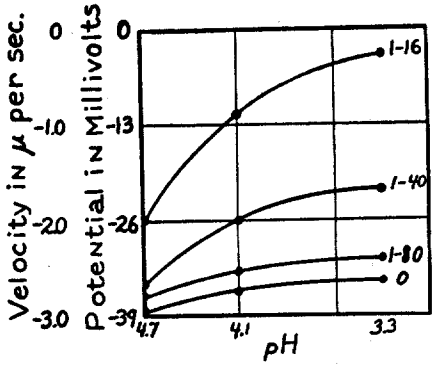

FIG. 3. The potential of washed but untreated *Bacterium coli* in 0.01 N lactate solutions to which "acid extract" has been added in a concentration indicated on the curves.

substance, probably a protein, derived from the bacteria themselves; this serves to stabilize the cell suspension, even when the cataphoretic potential is zero. Since Loeb (1922–23) has shown that true protective colloids, such as gelatin, maintain the stability of suspensions irrespective of the cataphoretic potential difference (by virtue of their higher attraction for the molecules of the solvent than for each other), it is probable that this protein acts as a protective colloid. When sedimented and suspended in fresh solutions, there is still enough of this protein adhering to the bacteria to act as a protective colloid and prevent agglutination. When this substance is removed by washing, the bacteria are now found to agglutinate at

more alkaline reactions than did the untreated bacteria. It is necessary to point out that bacteria that have been acid treated for a few hours, washed, and then placed in buffer solutions, may undergo still further changes in potential; while this fact has been verified in a preliminary way, it has not been studied in detail.

SUMMARY.

1. Stability and potential of *Bacterium coli* suspensions depend, not only on the strain of the organism and the medium in which it is suspended, but also on the previous treatment of the suspension, and the length of time it has been in the medium.

2. When treated at acid reactions, the negative charge on the bacteria is diminished; with some strains, a positive charge is acquired. Changes in stability accompany the changes in potential.

3. Washing acid-treated bacteria at neutral or slightly alkaline reactions does not restore the original potential; the zone of flocculation is moved toward the alkaline side.

4. These changes are due to two factors: the extraction of a soluble protein which combines with the surfaces of the cells, and a further irreversible change of the cell or its membrane.

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