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.

161 (2393)

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.

their size. The placques may vary in the same plate from the size of a pin-prick to that of a size larger than the head of the pin. A number of authors have succeeded in demonstrating that by isolating and propagating each of the variants one can obtain pure strains of "bacteriophage"—showing very little if any variation in the size or distinctness of the placques (or spots). In their opinion the co-existence in one plate of spots of different size is indicative of impurity of the lytic strain.

Having in our possession a strain of "bacteriophage" which showed a tendency to give rise to spots of lysis of various sizes, we considered it impure and attempted to isolate the several component strains by the usual method of daily transfers. When we found that pure strains of "bacteriophage" thus secured would unexpectedly show marked changes in respect to the average size of spots or their distinctness, we attempted to ascertain the cause of the changes. With this end in view we made a systematic study of the factors involved in the placque formation and made the following observations.

1. Within certain limits, the average size of the placque changes inversely with the number of bacteria susceptible to lysis present in each cc. of agar.

2. The average size of the placque depends upon the relative concentration of young and old individuals in the culture—the greater the number of old bacteria, the smaller the spots.

3. While the ratio between the number of resistant (capable of multiplying in the presence of bacteriophage) and susceptible bacteria has no marked effect upon the size of the spots, this ratio influences their distinctness. The greater the relative number of resistant bacteria, the less distinct are the spots. The difference in appearance between the two types of spots is particularly evident when observed under the low-power of the microscope, the less distinct spots often being indistinguishable from the surrounding growth.

4. The concentration of agar has a most marked effect upon the size of the spots. When the agar is more concentrated the spots are smaller.

Since we observed in another connection that the hydrogen ion concentration and the concentration of salt in the medium may affect the appearance of the plaques, we wish to state that in these experiments the pH of the medium was 7.4 and the only salt present was 0.4 per cent sodium phosphate.