Entirely analogous results were obtained with a *B. typhosus* phage exhibiting a secondary valency for *B. dysenteriae* "Flexner" and with *Staphylococcus aureus* phage, exhibiting a secondary valency for *Staphylococcus albus*. On the other hand when a polyvalent phage was produced by deliberately mixing several independent and unrelated phages (such as anti-coli and anti-typhosus or anti-staphylococcus) and the mixture was subjected to heating, some of the valencies lost during the heating could not be regenerated by the passage of the residual phage with a susceptible organism.

These experiments show, therefore, that when true multiple valencies are present in pure phages they are determined by some sort of a specific relationship among the susceptible bacteria perhaps remotely analogous but not identical with the relationships determining serological group reactions.

Incidentally, these results offer additional evidence against the parasitic nature of phage—for if phage were a parasite capable of invading *B. coli*, *B. dysenteriae* "Shiga", and *B. dysenteriae* "Flexner", it is difficult to see how heat could destroy its power of invading the 2 latter organisms while leaving the invasive power for the first unimpaired.

Furthermore, the subsequent recovery of lytic power for *B. dysenteriae* "Shiga" and *B. dysenteriae* "Flexner" can not be explained on the basis of "adaptation" of the parasite, since in these experiments the development of additional valencies by the monovalent anti-coli-phage took place as a result of the passage with *B. coli* only, and did not take place when *B. dysenteriae* "Shiga" or *B. dysenteriae* "Flexner" were exposed to it.

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Stimulation of Bacterial Metabolism by Bacteriophage.*

J. DOUBLY AND J. BRONFENBRENNER.

From the Department of Bacteriology and Immunology, Washington University Medical School.

Examination of an automatic cinematographic record of the progress of lysis of *B. coli* in the presence of bacteriophage suggested that addition of phage to pure cultures of *B. coli* causes an increase

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in the rate of cell division as compared with the rate of division maintained by the same cultures in the absence of bacteriophage.[1]

This growth stimulating effect of bacteriophage was again observed in a very striking way, when the phage and bacteria were brought together on solid medium under circumstances which precluded the free lysis of bacteria. Under these conditions the density of bacterial growth was markedly increased under the drops of bacteriophage.[2, 3] These and subsequent observations lead us to conclude that the effect exerted by phage is primarily that of acceleration of the metabolic activities of susceptible bacteria (including the increased rate of multiplication) and that actual lysis, while the most striking feature of the phenomenon, is in fact only secondary in importance and occurs only as a result of this change in the metabolic rate and the consequent osmotic changes in the cells taking place if free water happens to be available.[4, 5]

The increase in the rate of multiplication of bacteria in the presence of phage has been observed by many investigators. However, in most instances the evidence was more or less indirect. More recently there appeared two accounts of experiments in which the authors attempted to count bacteria directly and concluded that the addition of phage does not cause acceleration of growth.[6, 7]

In view of the manifest importance of this question in connection with the proper understanding of the mechanism of the transmissible lysis we decided to carry out further experiments.

Two flasks, each containing 200 cc. of broth (pH 7.4), received a suspension of bacteria so diluted that the initial count in the flask approximated 10^5 organisms per cc. To one of the flasks was added 2 cc. of active phage (the concentration of phage was varied in different experiments); the other flask serving as control received 2 cc. of the same phage inactivated by heat.

At intervals samples were removed from each flask and examined as follows: (a) A portion of the sample was introduced into a cup of a nephelometer and compared against an arbitrary turbidity

[1] Bronfenbrenner, J., Muckenfuss, R. S., and Hetler, D. M., *Am. J. Path.*, 1927, **3**, 562.

[2] Bronfenbrenner, J., and Hetler, D. M., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 480.

[3] Hetler, D. M., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 806.

[4] Bronfenbrenner, J., and Hetler, D. M., *Am. J. Path.*, 1928, **4**, 622.

[5] Bronfenbrenner, J., *J. Bact.*, 1930, **19**, 19.

[6] Eaton, M. D., *J. Bact.*, 1931, **21**, 143.

[7] Andrews, C. H., and Elfort, W. J., *Brit. J. Exp. Path.*, 1932, **13**, 13.

standard. The observations were recorded in arbitrary units of the nephelometer scale.

(b) A second portion of the sample was immediately transferred to a solid medium for the counting of the number of viable bacteria. The media used for this purpose were designed to arrest the progress of lysis. In some experiments 50% gelatine, in others 4% agar or a 2% agar containing 0.75% of sodium citrate, were used. (The latter was found less satisfactory.)

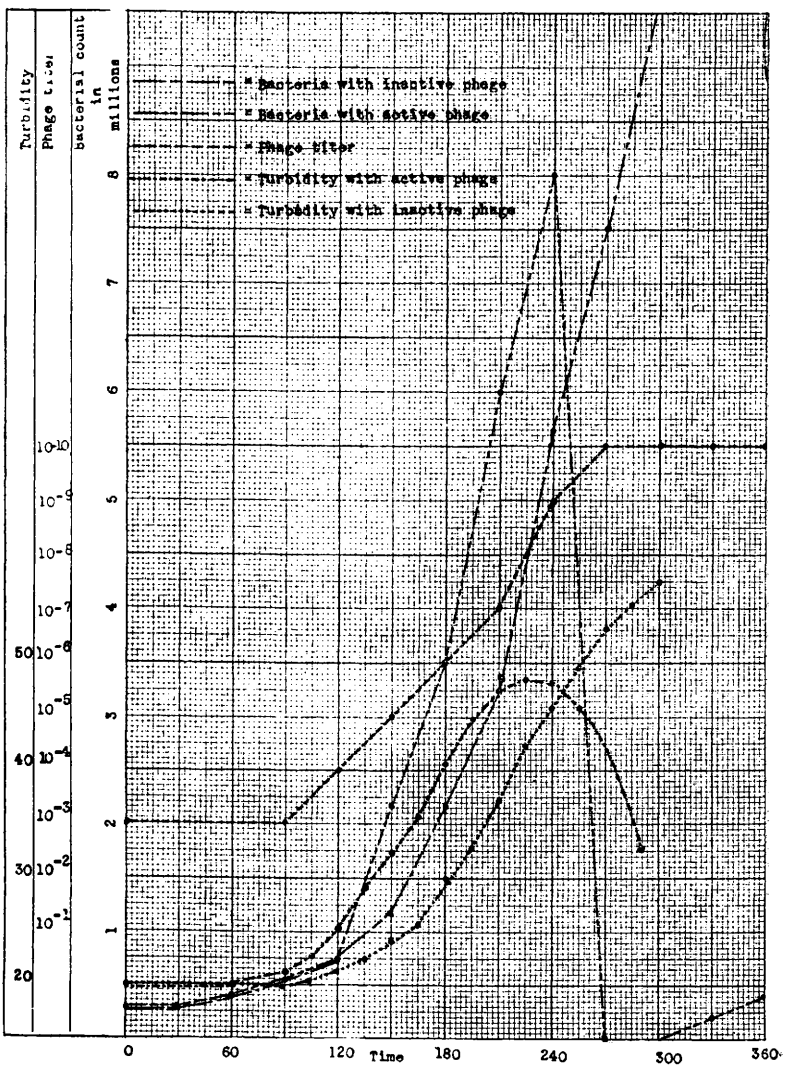

FIG. 1.

(c) A third portion of the sample was titrated for bacteriophage content.

As can be seen from a protocol of one of the experiments, the culture growing in the presence of active bacteriophage increases in turbidity faster than does the control culture. The curves representing the turbidity changes are in general similar to those representing the changes in bacterial count, except that acceleration of growth in the presence of phage as recorded by the increase in turbidity is apparent earlier than it could be detected by the bacterial count. This we believe is due to the fact that gelatine fails to completely stop all lysis and certain number of bacteria present in the samples undergo lysis after being transferred upon the plates. In spite of this inaccuracy of the method, the results show quite clearly that in the presence of phage the lag period is somewhat shortened, and that bacteria multiply at a greater rate than they do in the absence of phage. As the concentration of phages increases in the culture up to the critical titer (10^{-8} cc.) the massive lysis takes place.

These experiments substantiate by direct evidence our earlier observation that phage stimulates the metabolism of bacteria. In order to elicit this phenomenon, however, it is necessary to adjust the experiment in such a way that lysis of bacteria would be delayed, otherwise the lysis occurring simultaneously with growth of bacteria obscures the results. Essentially analogous results were obtained with *B. coli* and staphylococcus.

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Is Ant. Pituitary Hormone Demonstrable in Urine of Graves Disease, in Urine of Guinea Pigs Injected With Ant. Pituitary Extract?

MARGARET G. SMITH AND ELIZABETH MOORE. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine.

Loeb and Bassett[1] have shown that an acid extract of cattle anterior pituitary produces hypertrophy of the thyroid gland of guinea pigs. They noted that this experimentally produced hypertrophy characterized by an enlargement of the acinar cells, softening and absorption of colloid, and an irregular and often slit-like shape of the acini, and by the formation of papillary ingrowths into the

[1] Loeb, Leo, and Bassett, R., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860.