Dr. F. G. Cottrell, chemist and inventor of the precipitation process, laid fresh emphasis on the necessity of presenting science as news to newspapers.

Capt. J. F. Hellweg, of the U. S. Navy, spoke briefly on "what should not be printed."

Professor Knight Dunlap, of the Johns Hopkins University, contrasted conditions in science news reporting since Science Service entered the field with what they were before that time, and expressed the hope that this organization would continue its work independently, not only for the work it is doing itself but for its stimulating effect on the science reporting of the other newspaper syndicates.

Dr. W. H. Howell, of the Johns Hopkins Medical School, chairman of the executive committee of Science Service, closed the discussion with an expression of thanks to his fellow-scientists for their cooperation in the work of Science Service.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A CRITIQUE OF THE SERIAL DILUTION METHOD FOR QUANTITATIVE DETERMINATION OF BACTERIOPHAGE

THE two current methods for quantitative determination of bacteriophage are the plaque count and the serial dilution technique. The latter procedure involves testing successive dilutions of the lytic principle for ability to produce visible lysis of a broth culture of susceptible bacteria. The final effective dilution is assumed to contain at least one phage particle and the titre of the original lysate is calculated upon this basis.

For the plaque count, phage dilutions are plated upon a substrate of susceptible organisms. The resulting punched-out, bare areas in the surface growth are considered to represent the loci of single phage particles. Their number, together with the dilution factor, should theoretically furnish a simple means of estimating the total phage/ml. in the sample.

That agreement between the two methods above outlined is far from satisfactory for quantitative work is apparent from a survey of the literature. Further, the use of either procedure alone does not permit of accurate comparative determinations. In the case of the plaque count it has been pointed out by Bronfenbrenner [1, 2] that there are several factors not amenable to ready control which effect the formation of plaques; consequently, checks are difficult to procure.

The serial dilution technic presents similar limitations. Clark[3] has analyzed the method upon purely statistical grounds and concludes that with a dilution factor of 0.1 only 60 per cent. of parallel runs on the same solution should give an identical end-point. It is shown in the present paper that some of the difficulties encountered in practical application of the method are explicable on the basis of the kinetics of the bacterium-bacteriophage reaction.

The chief points established regarding the mechanism of phage action, as exemplified by susceptible Staphylococci growing in the presence of anti-Staphylococcus phage, may be briefly summarized as follows:

(1) Phage formation is conditioned by bacterial growth.[4]

(2) The percentage rate of increase in phage is proportional to the percentage rate of increase of bacteria, *i.e.*,

$$\frac{dP}{Pdt} = C\frac{dB}{Bdt.}$$

(3) Phage accumulates within the bacteria, meanwhile maintaining equilibrium with phage in the broth outside the cells, until a certain concentration of phage per bacterium is attained, when lysis ensues. There is thus a definite lytic threshold.[4, 5]

(4) Phage is distributed between susceptible cells and the fluid medium in two ways depending upon whether the bacteria are alive or dead. With live cells (resting or growing) distribution is of normal type and diffusion of phage, into or out of the organisms, proceeds according to a definite quantitative relationship. If the cells are dead, however, they adsorb phage irreversibly and equilibrium may be represented in terms of the Freundlich adsorption isotherm equation.[6]

The purpose of the serial dilution procedure is to ascertain the highest effective phage dilution capable of initiating visible lysis in the test suspension and consequently the technique has been assumed to rest upon a qualitative test for the presence of phage. However, in the case of the organism and phage studied, the qualitative test is conditioned by definite quantitative factors and in effect does not determine whether phage is present or absent in the higher dilutions but rather whether or not a certain minimum quantity of phage is present. This amount is not constant but varies with test conditions.

Consideration of two cases will clarify the above statement. Keeping in mind the dependence of lysis upon development of a certain high intracellular con-

[1] J. Bronfenbrenner and C. Korb, *J. Exp. Med.*, 42, 483, 1925.

[2] J. Bronfenbrenner and C. Korb, *Proc. Soc. Exp. Biol. and Med.*, 21, 315, 1924.

[3] H. Clark, *J. Gen. Physiol.*, 11, 71, 1927.

[4] A. P. Krueger and J. H. Northrop, *J. Gen. Physiol.*, 14 (No. 2), 223, 1930.

[5] J. H. Northrop and A. P. Krueger, *J. Gen. Physiol.*, (in press).

[6] A. P. Krueger, *J. Gen. Physiol.*, 14 (No. 4), 493, 1931.

centration of phage per bacterium, it follows that with a large initial concentration of phage ($[P]_o$), the initial concentration of bacteria ($[B]_o$) may be varied within rather wide limits and lysis will still occur. That is, relatively few cell divisions will result in the production of enough phage to raise the phage-bacterial ratio to the lytic level. This has been demonstrated experimentally.[4]

On the other hand, if $[P]_o$ is small as must obtain in the last few tubes of the serial dilution set-up, it is clear that the magnitude of $[B]_o$ will considerably influence the outcome. A certain minimal number of cell divisions will be required to raise [P] to the effective threshold for lysis and if $[B]_o$ is too large the organisms will enter the maximal growth stationary phase before such a condition is realized. For a given small $[P]_o$ there thus exists a maximal $[B]_o$ beyond which lysis can not be expected to occur. Again, experimental confirmation is direct. With $[P]_o$ small and constant in a series of tubes, successively larger $[B]_o$'s are added under conditions described in previous work.[4] Cellular dissolution results in all the lower members of the series up to a certain point beyond which the suspensions do not clear.

That phage may have been present in the uncleared tubes can be demonstrated by filtering these suspensions and again seeding them with identical $[B]_o$'s. Frequently two or three of the second series will lyse, indicating that phage was originally present but in such small amounts that the lytic threshold was unattainable. However, bacterial reproduction resulted in an appreciable increase in [P] (proven by direct titration) and the second passage upon susceptible organisms developed a concentration adequate for lytic action.

Since development of the lytic end-point depends upon the initial concentration of bacteria used in seeding the test series, the serial dilution technique as usually carried out presents a potential source of error. The customary assumption that the final effective dilution contains one phage particle and the unlysed tubes no such particles is substantially in error and it should be recognized that the titration data can not be expressed accurately in terms of actual lytic particles. Most work does not necessitate absolute enumeration. Nevertheless, in order to make even comparative quantitative determinations of phage, $[B]_o$ should be carefully controlled.

At least one other factor significantly influences the test; namely, the percentage of dead bacteria present in the suspension. Dead cells adsorb phage quickly, irreversibly and in relatively large amounts under ordinary test conditions.[6] Hence their presence will delay, and may readily prevent altogether, the development in live cells of the critical lytic P : B ratio. Dead cells can not be entirely excluded, but it is rational to limit their numbers to a small and rather constant percentage of total cells by employing young cultures of the same age.

The writer has described a method for comparative quantitative phage determinations possessing none of the objections to which the plaque count and serial dilution technique are open.[7, 8] The procedure is based upon a relationship between $[P]_o$ and the time of lysis predicted by kinetic analysis of the bacterium-bacteriophage reaction.[4] Experience with over 200 routine titrations has demonstrated that [P] can be determined with an accuracy of $\pm$ 3 per cent.

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A SIMPLE METHOD OF REARING AND MOUNTING HOOKWORM[1] LARVAE

IN an effort to partially meet the demands for suitable and adequate laboratory materials for students in biology, the writers have developed a simple method of rearing and mounting hookworm larvae. While the procedure is in part a modification of older methods the technique is very simple, requiring little or no special equipment, and is thus an improvement over the older methods. It is possible by this method to supply large numbers of students with living and mounted specimens of this important human parasite with a minimum amount of trouble to the instructor. This is especially true in the Southern states, where hookworm reservoirs are relatively easy to locate.

REARING

The usual method of mixing infected feces with powdered charcoal in the proportions of approximately two parts of charcoal to one part of feces was used in rearing larvae. Both animal and plant charcoal were used with equally good results. Suitable containers, such as flat-bottomed watch glasses, were filled with the charcoal mixture and placed in larger shallow pans containing water about one fourth of an inch deep. Each pan was then covered with a plate of glass and the material was incubated at room temperature until the larvae reached the desired stage.

Two general methods of isolating larvae were used. In the first method the material remained undisturbed for a week or ten days until many of the infective larvae had migrated from the fecal material into the surrounding water. The water was then strained through several layers of cheese-cloth into a specially arranged funnel, and the lower contents of the fun-

[7] A. P. Krueger, *J. Gen. Physiol.*, 13, 557, 1930.
[8] A. P. Krueger, SCIENCE, lxxii, 1872, 507, 1930.
[1] *Necator americanus.*