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.*

nel drained off after a period of 6 to 10 hours. The second was a modification of Baerman's[2] method to permit the isolation of larvae at any stage of their development. In this procedure a heavy wire was bent into a circle of such diameter as to fit into a large funnel and rest on the sides about two inches from the top. Four layers of cheese-cloth were stretched over the wire ring with the edges of the cloth raised so as to form a crude flat bottomed bag. The watch glasses containing the fecal material were inverted on the bottom of the bag; it was then fitted into the funnel which contained enough water to cover the watch glasses. The funnel was equipped with rubber tubing and a stopcock at the lower end and was mounted on a suitable stand. This arrangement of the funnel was the same as that referred to in the first method of isolation.

The larvae that collected over night in the stem of the funnel were drawn off through the stopcock into vials about one inch in diameter and two or more inches deep. The larvae rapidly settled to the bottom and could be obtained in large numbers for classroom study or for mounting. The writers, using a single drop of material, have observed more than forty individuals in the same field of a 100-magnification compound microscope.

Vials of living larvae were kept in the laboratory for about three weeks; they could be kept for longer periods. It was necessary, however, to pipette most of the water off and replace it with fresh water daily to prevent the larvae from dying.

MOUNTING

In preparing larvae for mounting, alcohol was used as a killing and hardening agent. Most of the water was drawn out of the vial and the vial filled with 5 per cent. alcohol. After standing fifteen minutes, the 5 per cent. alcohol was replaced with 70 per cent. alcohol. The larvae were hardened within two hours and remained in this solution, in good condition, for more than two weeks.

The larvae were mounted by transferring a drop of material from the bottom of the vial to a slide. The alcohol on the slide was immediately ignited and allowed to burn off, thereby affixing the larvae. The mount was then placed under a pair of binoculars and any large pieces of débris were removed with a needle, after which the larvae were covered with Delafield's Haematoxylin. After staining 10 to 15 minutes, the excess haematoxylin was flushed off with water running slowly from the tap; only a few seconds were required for the washing. The slide was again placed under the binoculars to see that the desired depth of color had been obtained, and was allowed to dry thoroughly. The larvae were then mounted under balsam in the usual manner.

Congo red, Orange G and alum cochineal were also used successfully as stains, but the larval structures were more distinct when haematoxylin was used.

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SPECIAL ARTICLES

STIMULATIVE EFFECTS OF ILLUMINATING GAS ON TREES

IN an investigation to determine significant symptoms of illuminating gas poisoning of shade trees a series of stimulation phenomena have been observed and recorded during February and March, 1932. The general features of these responses will be described briefly at this time since they may have application during the early spring in the detection of shade trees undergoing the incipient stages of illuminating gas injury.

In the investigation so far, no attempt has been made to determine how small an amount of illuminating gas will give responses in the plant material used. Entire potted trees, tree buds, tree roots, cuttings of shrubs and tree seeds have been subjected to complete atmospheres of illuminating gas and to atmospheres containing 10 per cent. to 40 per cent. of this gas by volume. The commercial product of the New Haven Gas Light Company was used throughout. This gas is a mixture of coke-oven gas and water gas.

When small potted dormant black oak, red oak and catalpa trees were subjected in a closed ash can to an atmosphere containing approximately 20 per cent. of illuminating gas for 24 and 48 hours, respectively, and then placed in a greenhouse, the buds of the gassed trees began to swell and the leaves to unfold weeks before similar control trees showed any bud activity. The exact gain in time cannot be stated as yet because the control trees at this time (a period of 4 weeks) are still dormant.

When test-tubes filled with illuminating gas were sealed over the dormant terminal buds of potted red and black oak trees for one and two days, respectively, active bud development and foliage production was observed weeks in advance of these responses in control trees. The buds of the control trees were en-

[2] G. Baerman, "Über Ankylostomiasis deren Ausbreitungsbedingungen durch die Bodeninfection und deren Bekaempfung." Geneeskundig Tijdschrift voor Nederlandsch-Indie, 57, 579–673, 1917.