microscope. For laboratory experiments where a high degree of accuracy is not essential the tube is graduated in millimeters, the variations in the cross-section of the capillary usually being very small. The volume of the capillary per centimeter is determined with a mercury bead. Changes in volume can be determined with accuracy as small as .01 cu. mm. of water when the capillary is accurately graduated.

Since the apparatus described was first made, Svedberg[5] has measured the hydration of gelatin by means of a dilatometer in which water and paraffin oil are used. The gelatin is held on a disc suspended by a wire which passes up through the capillary tube. After constant temperature is attained the gelatin is lowered by means of the wire into the lower layer of water. After the hydration of the gelatin the disc is raised to its former position. In this method there would be considerable chance of error. If the substance were wet by paraffin oil, hydration in many cases would be inhibited if not entirely prevented. Also corrections for the volume of the wire in the capillary would be necessary.

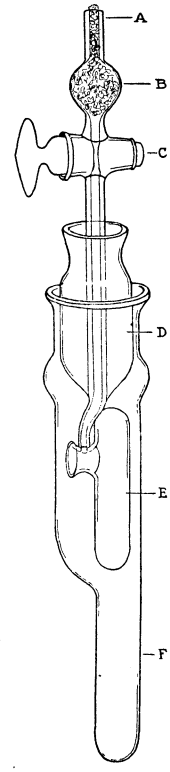
H. C. HAMPTON

PLANT PHYSIOLOGY LABORATORY,
UNIVERSITY OF WISCONSIN

SPECIAL ARTICLES

DOES BACTERIOPHAGE RESPIRE?

AS bearing on the discussion of the nature of bacteriophage, namely, whether it is a living virus or not, I reported failure to detect evidences of respiration such as should have occurred had the bacteriophage been a living organism.[1] The experiments were performed in a respirometer in which an initial volume of CO_2-free air was sent along a closed circuit[2] by means of a double action mercury pump, through a vessel containing the material which was to be studied. The production of CO_2, on the assumption that all volatile acid consisted of it, was estimated colorimetrically by the method of Ray.[3] In view of the failure to obtain evidence of respiration by bacteriophage, the question arose as to whether respiration had not been suppressed by mercury vapor, accumulating while the air was repeatedly passing through the pump. Although bacteria and tissue emulsions placed in the apparatus were not prevented from respiring and producing CO_2, the possible action of mercury had still to be considered, especially in view of the fact that while bacteria and tissues remained in the respirometer for minutes, or hours only, bacteriophage remained there for four days.[1] This difficulty has been overcome by using a recent suggestion of Parker,[4] namely, shaking of the respirometer in order to secure even distribution of gases. The method, as now employed, can, we believe, be advantageously used for the study of respiration of various micro-organisms, including the filtrable viruses, and in atmospheres of oxygen or of inert gases, at any desired pressure up to atmospheric.



The apparatus is shown in the diagram. It is loosely assembled (C and D), plugged with cotton (B), and sterilized by dry heat. After removal from the oven, a measured amount of bicarbonate and indicator[3] is run into the outer tube (F), and the material to be examined is placed in the inner tube (E), the ground joints (C and D) are smeared with sterile vaseline, and the parts pressed together, due precautions being taken to prevent bacterial contamination. The apparatus is now connected (at A) through a two-way stop-cock with a vacuum pump and with a wash bottle containing caustic soda, and delivering air free of CO_2, or an inert gas, and while being rocked is alternately exhausted with the pump and filled with CO_2-free air, until the color of the solution in the outer chamber (F) reaches that of the stand-

[5] *Jr. Amer. Chem. Soc.*, pp. 2673, Dec., 1924.

[1] Bronfenbrenner, J., *Proc. Soc. Exper. Biol. and Med.*, 22, 81, 1924.

[2] Osterhout, W. J. V., *Jour. Gen. Physiol.*, 1, 17, 1918.

[3] Ray, G. B., *Jour. Gen. Physiol.*, 6, 509, 1923.

[4] Parker, G. H., *Jour. Gen. Physiol.*, 7, 641, 1925.

ard. (In order to maintain the sealing of the joints it is better, when possible, not to bring the pressure within the respirometer fully up to the atmospheric.) The aeration is now discontinued, but the rocking kept up. The rate of CO_2 production is recorded in terms of time necessary to effect a definite change of color of the bicarbonate (in F), or by allowing a definite time to elapse, and comparing the color of the bicarbonate attained at that time with a set of standards. The computation of the CO_2 resulting is made in a manner described by Ray.[3] In certain instances a part, or even all the CO_2 produced, is consumed in producing carbonates, in which cases, after the experiment has proceeded for a certain time, one removes the cotton plug and, having filled the bulb (B) with acid, permits a small amount of the acid to enter the inner chamber (E), thus liberating the CO_2. At this time rocking is resumed and continued, until the color of the indicator no longer changes. The actual amount of CO_2 produced will be represented by the difference between the total CO_2 liberated in two succeeding determinations, the first serving as a control for the second.

In the case of the bacteriophage, the last of the steps described was omitted, since it was found possible to acidify the solution (up to $CH = 3 \times 10^{-5}$), before placing it in the respirometer, without affecting the activity of the bacteriophage. The acidified bacteriophage solution proved to be as potent at the end as at the beginning of the test.

A large number of experiments was made with different types of bacteriophage, and in none was any indication of CO_2 production obtained.

For the study of anaerobic respiration, 1 per cent. of glucose and a drop of indicator[5] are added to the bacteriophage solution before it is placed in the respirometer. Should production of non-volatile acid occur, it will be indicated by the change in color of the bacteriophage solution itself. The change in color in the outer tube indicates the production of volatile acid. In the case of bacteriophage there is no change in the color of the indicator in either chamber.

J. BRONFENBRENNER
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, NEW YORK

INHERITANCE OF ANTHOCYANIN IN CREPIS

A REDDISH purple pigment assumed to be anthocyanin is present in varying degrees in plants of all the species of Crepis which have been examined. Selection in *C. capillaris* (L.) Wallr. gave races differing considerably in the amount of pigment present, but no plants have appeared in which anthocyanin was completely absent. When races differing in the amount of pigment were crossed, the F_1 and F_2 results were those generally described as due to a number of genes conditioning the appearance of a single character. Two selected inbred strains appeared to differ in only two genes, giving a 9 to 7 ratio in F_2.

In view of our results regarding this pigment in *capillaris*, the discovery in 1923 of a single pure green plant in a culture of *C. Dioscoridis* L. is noteworthy. When crossed with the common pigmented type, the F_1 was fully pigmented. The following data from F_2 cultures indicates this difference between pigmented and non-pigmented is conditioned by a single gene:

Culture No.	Purple	Green	Total
25.12	15	4	19
25.14	19	7	26
25.15	69	27	96
Total	103	38	141
Calculated	105.75	35.25	141
Difference		2.75 ± 3.47	

The amount of anthocyanin varies considerably in different plants of *Dioscoridis*. No attempts have been made to select extreme races comparable to those secured in *capillaris* to determine whether the different degrees of pigmentation are determined by different genes affecting this character. The results in *Dioscoridis*, however, show that one main gene conditions the production of any pigment. There are probably modifying genes that influence the amount of anthocyanin which a plant may develop in the presence of the main pigment-determining gene.

The single green plant among twenty plants of the 1923 culture was evidently the result of a gene mutation, but whether it occurred in a single gene of some ancestral plant and appeared after the chance union of two gametes carrying the recessive gene or whether it occurred after the fertilization which produced this particular plant is hard to determine. Progenies from purple sibs of the green plant failed to produce any more green plants, as would be expected if it had appeared as an extracted recessive.

No difference could be observed between the development and vigor of the green plants and those having anthocyanin.

J. L. COLLINS
UNIVERSITY OF CALIFORNIA

[5] Bronfenbrenner, J., *Jour. Med. Res.*, 39, 25, 1918. (This indicator is preferred because it is less subject to reduction.)