of pictures. To the astronomer, the photographic surveyor and to those engaged in photographic research it will be invaluable.

P. G. NUTTING

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A DILATOMETER FOR MEASURING THE HYDRATION OF COLLOIDS

THE hydration of colloids is an important phenomenon in many physiological processes, as well as in many non-living colloidal reactions. Thus it is of interest to be able to measure the rate and amount of hydration and possibly, in some instances, to estimate the forces involved in the process. The swelling of the colloidal material usually occurs to a measurable extent as a result of hydration. The increase in size has long been used as a measure of the extent of hydration. Other methods which have been used with more or less accuracy are by (1) direct measurement of the pressure necessary to prevent swelling; (2) measurement of the heat of hydration; (3) measuring the rate of increase in weight of the material during hydration; (4) determining the increase of the specific gravity of the material; (5) determining the change in the freezing point of a solution of known concentration in which the material is immersed, and (6) determining the change of the specific gravity of a known solution of alcohol or salt in water in which the material is immersed. All these methods, however, are limited in their application or subject to large experimental errors, which can not be measured. Some colloidal substances do not swell equally in all dimensions, thus, any linear measurement is not an accurate measure of the extent of hydration. It is also known that the rate of hydration varies inversely to the degree of saturation, thus the total amount of hydration largely depends upon the degree of dryness of the colloidal material. An apparent increase in volume may also be brought about by an increase in dispersion, disintegration of the colloidal aggregates, which is often accompanied by a decrease in degree of hydration[1] as, for example, when a gelatin hydrogel changes to a hydrosol, or solid starch grains form a hydrosol when heated. A portion of the water held by a colloidal substance may be held by surface tension and thus not be water of hydration.

Hydration is regarded as a physical, chemical or physico-chemical union of water with many colloidal and non-colloidal substances. In any case, there is either a physical or a chemical condensation of the water; as in cases where there appears to be a purely physical relation the water is held in a highly compressed condition on the surface of the colloidal particle by the force of attraction between the two substances, or hydration is a phenomenon of solid solution; or there may be evidence of a chemical change, the water being held in a weak chemical union by the molecules of the substance.[2] If the colloid is a protein, some believe that the ions of the protein molecules are the units which are hydrated.[3] Thus a condensation or decrease in the volume of the water during the process of hydration furnishes an accurate measure of the rate and extent of the hydration. The volume of condensation of water at saturation in cubic millimeters per gram of dry material is here termed the "hydration factor" which is characteristic of the material under the conditions of the determination.

Some fraction of the water absorbed is held in a non-condensed condition and is not to be considered as a part of the water of hydration. This fraction varies with the conditions and the kind of material used. It is often impossible to measure the absolute amounts of water of hydration and water held in a non-condensed condition, but for comparative purposes the relative amounts of each are represented by the ratio of the hydration factor to the total amount of water absorbed per gram of dry material. This relation is termed the "hydration ratio." Thus the hydration ratio indicates the relation between the amount of water of hydration and the amount of the more mobile supply of water held in a non-condensed condition. It is probable that the water of hydration has the more important function in physiological processes and the water held in a non-condensed condition is utilized in hydration as these processes continue.

With some substances, as starch, for example, the absolute amount of water held in a non-condensed condition may be measured by the increase in weight of the dry material after it has attained equilibrium with air saturated with water vapor. In such cases, from the amount of water of hydration per gram of dry material and the hydration factor, the decrease in volume of a unit volume of water is easily calculated. The pressure necessary to cause an equal decrease in volume of pure water may be determined by referring to tables of the volume of water at dif-

[1] Fischer, Martin, and Coffman, W. D., *Jour. Am. Chem. Soc.*, 40: 304, 1918; Fischer, Martin, "Soaps and Proteins," New York, pp. 219, 1921.

[2] Loeb, J., "Proteins and the Theory of Colloidal Behavior," pp. 194, 1922.

[3] Jones, H. C., *Am. Chem. Jour.*, 34: 291, 1905.

ferent temperatures and pressures[4] and possibly is equal to the force of hydration. This is based on the assumption that the decrease in volume of the water results on account of the force of attraction between the water and the material.

Figure 1 is a diagram of the apparatus which has been used in this laboratory and has proved particularly interesting as an experiment to demonstrate the condensation of water in the process of hydration. To measure the rate of hydration of a colloidal substance the bottle is filled about two thirds full of dry mercury. A small amount of the material, which has been thoroughly dried, is placed on the surface of the mercury. The rubber washer is placed on the rim of the neck of the bottle. The hard rubber stopper which supports the nickel disc and capillary tube is then inserted in the neck of the bottle and the material is pushed under the surface of the mercury below the disc. The bottle is then immersed in water. When it is entirely filled, including no bubbles, the stopper is pressed tightly down upon the rubber washer. The lid is then slipped over the upper end of the capillary tube, the clamp placed with its two ends under the collar which surrounds the neck of the bottle. The apparatus is made water-tight by tightening the screw which at the same time raises the surface of the water in the tube. The apparatus is then placed in an accurately controlled thermostat until a constant temperature is attained. The water level in the tube is adjusted to the zero mark at the top of the tube by tightening the screw. The apparatus is then tilted until the dry material slips from under the nickel disc and rises to the surface of the mercury, coming in contact with the water without any change in the total volume of material in the bottle. As hydration proceeds the surface of the water in the tube lowers until saturation is reached, which requires varying lengths of time depending upon conditions and material used. If the material is non-porous, the decrease in the volume of water is on account of the condensation accompanying hydration. If the material is porous and some air is present with the colloid a small bubble may be displaced and rise to the surface of the water in the bottle. In this case a small error is unavoidable, owing to the slight change in pressure on the gas. However, the air may be removed by suction through the capillary tube by means of a vacuum pump. Number 13 of Figure 1 represents an improved disc which allows the material to be displaced from under the disc with only a slight tilt of the apparatus. The disc, 13, rotates clockwise on the axis, 14. As the apparatus is tilted clockwise the disc will turn up owing to the pressure of the mercury, thus allowing the material to rise readily into the water.

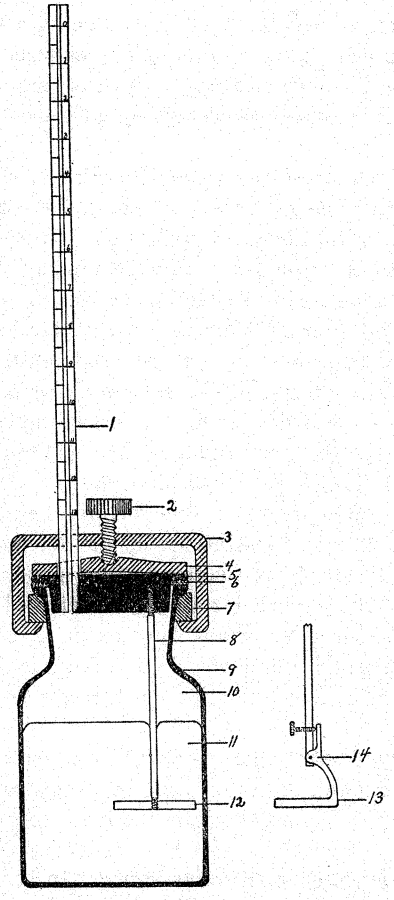

FIGURE 1

1. Calibrated capillary tube. 2. Brass screw. 3. Brass clamp. 4. Brass lid. 5. Hard rubber stopper. 6. Rubber washer. 7. Brass collar. 8. Nickel wire. 9. Wide mouth bottle of about 100 cc. capacity. 10. Water. 11. Mercury. 12. Nickel disc. 13. Improved nickel disc.

The method above described is particularly suitable for studying the effects of substances in solution upon hydration. When the material is a powder, such as starch, it is necessary to place it in a shallow capsule having one side open. Such capsules are easily made by dipping the end of a glass rod in collodion. After the ether has evaporated, dip the end of the rod in water before the collodion has entirely hardened. The collodion capsule can then be slipped off from the rod and dried. When thoroughly dry they imbibe very little water. The capillary tube can be accurately calibrated with a bead of mercury and fractional parts of the graduations are accurately made with an eye-piece micrometer in a horizontal

[4] Bridgman, P. W., Proc. Amer. Acad. Art. Sci., XLVIII, pp. 338, Sept., 1912.

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.

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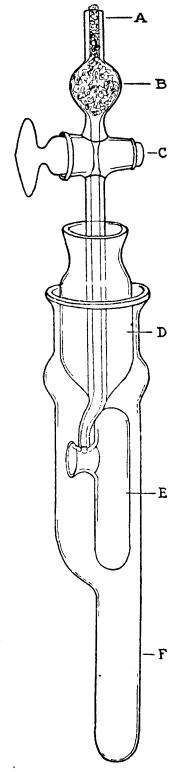
SPECIAL ARTICLES

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.