XIII. THE PREPARATION OF SILICA JELLY FOR USE AS A BACTERIOLOGICAL MEDIUM.

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DURING the course of experiments involving the cultivation of seedlings in nitrogen-free media, a supply of silica jelly was required, free from combined nitrogen and of such consistency that it would support the growth of seedlings in tubes.

Preparation of the jelly according to published methods [Smith, 1905] failed to give satisfactory or uniform results, especially with regard to the consistency of the gel, although great care was taken at every stage of the preparation. Since other workers seem to have experienced similar difficulties, it was thought that an attempt to standardise the method of preparation of silica jelly might prove useful and possibly save much labour. The present note is the outcome of such an attempt and is published in the hope that it may be of service to other workers.

Preliminary work showed that the critical stages occurred during dialysis and that it was necessary to work with membranes of permeability varying within certain limits in order to obtain a uniform gel after autoclaving.

Collodion membranes, prepared and graded, as described by Brown [1915], were tried for dialysing, but it was subsequently found easy to prepare membranes of the requisite permeability without alcoholic treatment.

A solution of collodion is prepared by dissolving 6 g. of clean white guncotton in 100 cc. of a mixture of equal parts of absolute alcohol and ether. A quantity of not less than 500 cc. is made up and is allowed to stand for at least 24 hours to dissolve. The solution is then decanted into a bottle of convenient size and shape for holding in the hand. Should minute insoluble particles be noticed on the side of the wetted flask, due to unequal nitration of the guncotton, the solution is decanted through a filter of glass wool. These insoluble particles are the cause of fragile and leaky membranes. Filtered collodion is always more satisfactory provided that precautions are taken to prevent undue loss of ether by evaporation. It is impracticable to prepare a solution of collodion of definite composition, but for the purpose of making membranes it should be of a consistency similar to that of a heavy oil.

Test tubes $7\frac{1}{2} \times 1\frac{1}{2}$ inches were found most serviceable for the work. They must be thoroughly clean and dry, and also smooth; tubes with projections on the glass give rise to leaky membranes.

A test tube is held in a slanting position in the hand and slowly rotated while the collodion is poured down the inside until it is completely coated and a depth of about one inch has collected at the bottom. The collodion is then poured *slowly* back into the bottle, the tube being rotated as before. As soon as all the mobile collodion has entered the bottle, the tube is quickly inverted and its mouth placed firmly on a piece of paper with a folded duster, or pad of some kind, underneath, so that the tube is completely closed and an ethereal atmosphere maintained inside while it is allowed to drain for exactly 6 seconds. The accumulation of collodion is quickly wiped off and the membrane rapidly dried by passing a glass tube, attached to a filter pump, up and down the test tube, which is rotated horizontally, meanwhile, to keep the coating of collodion evenly distributed. During this drying process the finger is lightly pressed against the collodion a little way inside the mouth of the tube every eight or ten seconds. Immediately the collodion ceases to adhere to the finger the test tube is quickly and carefully filled with water, and allowed to remain full for at least three minutes to prevent further loss of ether.

It is important that the drying process should not be carried too far; the object in view being to obtain a thick membrane of low permeability; it may take any time from 30 seconds to 4 minutes according to the temperature and humidity of the laboratory.

The membrane is now detached from the mouth of the tube by the aid of a needle and removed by allowing water to pass between it and the glass aided by gentle pressure of the fingers.

These membranes are slightly milky in appearance, *i.e.* of the ether rather than the alcohol type, and are sufficiently strong for easy manipulation. They must be kept under water and never allowed to become dry.

For the dialysis of silicic acid a membrane of this size, when filled with distilled water and suspended in air, should not allow the water to drop oftener than once in twelve seconds. Membranes have been successfully used, with little difference in the time taken for dialysis, which had a range of from 1 drop in 12 seconds to 1 drop in 80 seconds; but the thicker membranes are more reliable and may be used many times.

Pure re-fused sodium silicate was used for the preparation of nitrogen-free media. This is supplied in opaque lumps which are slowly soluble in boiling water, but are readily brought into solution when raised to a temperature of 140° for one hour in the autoclave. 100 g. of sodium silicate in a litre of distilled water is readily diluted to give a solution of specific gravity 1.09.

A standard solution of re-distilled hydrochloric acid of specific gravity 1.10 is now prepared. It requires approximately 530 cc. of distilled water to the litre of acid to reduce the concentrated acid to this density.

Equal volumes of the acid and silicate solutions are taken, the former is poured into a conical flask and the latter into a large separating funnel and dropped into the acid which is shaken meanwhile. This acid mixture is allowed to stand from three to four hours, according to the temperature of the laboratory, in order that the necessary chemical reaction may take place, the longer period being given when the temperature is below 15° . The mixture is then poured into a separating funnel and thence into the membrane, which is supported in a vessel of water to avoid undue strain. When nearly full the neck of the membrane is drawn together and secured with a rubber band and the membrane suspended in a gas cylinder—or other suitable vessel—of distilled water to dialyse. The distilled water is changed hourly during the day and dialysis continued until the silicic acid is neutral to litmus. This usually takes two days. After the first twelve hours the water in the dialyser is tested with litmus paper before each change, which gives an indication of the state of acidity of the solution inside the membrane and avoids unnecessary manipulations.

Running tap water as supplied to the town of Reading was repeatedly tried for dialysis, but always caused the silicic acid to gel in the membrane before dialysis was complete.

When neutral to litmus, the silicic acid is poured into a beaker, the nutrient salts added in concentrated solution, and the medium boiled for five minutes to expel air. If not sufficiently boiled the plugs will be blown out of the tubes in the autoclave and an uneven gel will result. The medium is "tubed" whilst hot and immediately autoclaved for 20 minutes at from 135° — 140° .

Silicic acid prepared in this way forms a gel on autoclaving; if slants are required the tubes must be placed in the requisite positions in the autoclave as the gel once formed is irreversible.

The success of the process depends upon:

(1) The use of a membrane of standard permeability. A rather thick membrane of low permeability gives the best results.

(2) A sufficiently long period being given for the sodium silicate and hydrochloric acid to react after mixing. This is a critical factor.

(3) The use of distilled water for dialysing. Constant changes of water especially in the earlier stages of dialysis in a vessel just large enough to take a membrane, are best.

(4) Tubing the medium as quickly as possible after removal from the membrane and immediate autoclaving. The autoclave should be heated up while the medium is being tubed.

If it is not convenient to tube and autoclave the medium immediately after dialysis, it may be kept for about 24 hours in the dialyser. If allowed to stand for a short time in a beaker after boiling, dialysed silicic acid will probably form needle-shaped crystals.

It should be noted that compounds of silicon form deposits on glass.

These deposits are difficult to remove. It is therefore advisable to restrict the use of glass apparatus within reasonable limits during the preparation of the jelly.

In conclusion I wish to thank Dr M. C. Rayner for suggesting this work, and also for giving me much valuable advice and criticism.

REFERENCES.

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