IN THE LABORATORY

Three-dimensional Modeling of Cyclones in Elementary Meteorology

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Students in elementary meteorology have more or less trouble in visualizing wave cyclones in three dimensions. For the past 6 years the writer has found helpful a laboratory exercise based on the use of a sheet of plasticine as the boundary surface between the air masses involved. In addition to the plasticine sheet ($\frac{1}{4}'' \times 10'' \times 16''$ is a convenient size), each student needs some small blocks of plasticine to prop up the main sheet, a knife, a few ticket pins with paper arrows colored to represent the cold and warm winds, a sheet of corrugated cardboard for the ground surface, and chalk to draw successive positions of the fronts on the "ground."

Given this material, the student proceeds to develop the successive stages in the standard Bjerknes wave cyclone "model." After the plasticine has been blocked up at a reasonable angle for a straight stationary front, the wave can be initiated and excess plasticine trimmed out so that the boundary sheet is in continuous contact with the ground. As the warm sector enlarges, additional trimming is done. When the occlusion stage is reached, the plasticine is split a sufficient distance up from the ground junction of the warm and cold fronts and one side tucked under. Thus, from the same cut the plasticine can be shaped to show either a warm or cold front type occlusion. Experience has shown that, once a student has gone through this entire sequence himself, he understands the structure of wave cyclones far better than if he were to rely on the standard cross sections alone.

Isolation of Crystalline Desoxyribonuclease From Beef Pancreas[1]

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A crystalline protein having a powerful desoxyribonuclease activity has been isolated from fresh beef pancreas by the method outlined below. Specific enzymatic activity of the protein is not diminished on recrystallization.

(1) *Preliminary purification by a modified McCarty's procedure* (*2*). An acid extract of ground beef pancreas, prepared according to the method of Kunitz and Northrop (*1*), is brought to 0.2 saturation of ammonium sulfate (114 gm of the salt/liter of extract). The solution is filtered with suction on Eaton and Dikeman No. 642 paper with the aid of 10 gm of Celite 503 (Johns-Manville Corporation) and 10 gm of standard supercel/liter of solution. The clear filtrate is then brought to 0.4 saturation of ammonium sulfate (121 gm/liter) and refiltered with suction, with the aid of 3 gm of Celite 503/liter, on Eaton and Dikeman No. 612 paper. The filtrate can be utilized for the preparation of chymotrypsinogen, trypsin, and ribonuclease. The residue, including the Celite, is suspended in 5 times its weight of water. The suspension is brought to 0.3 saturation of ammonium sulfate (176 gm/liter of water) and refiltered with suction, the filtrate then being discarded.

(2) *Incubation at 37° C followed by fractionation with ammonium sulfate.* The residue is suspended in 10 times its weight of water, and the suspension is brought to 0.15 saturation of ammonium sulfate (83.7 gm of salt/liter of water). The solution is titrated with about 2 ml of 5 N H_2SO_4/liter to pH 3.2 (glass electrode), heated to 37° C, and incubated for 1 hr at that temperature, after which it is cooled to about 20° C and filtered with suction with the aid of an additional 5 gm of Celite/liter of suspension. The residue is discarded.

The filtrate is titrated to pH 5.3 with 5 N NaOH (about 2 ml/liter) and brought to 0.5 saturation of ammonium sulfate (220 gm/liter). The precipitate formed, designated as "0.5 s.a.s. precipitate," is filtered with suction with the aid of 5 gm of Celite/liter of solution.

The clear filtrate is titrated with a few drops of 5 N H_2SO_4 to pH 4.0 (green to bromcresol green on test plate) and brought to 0.7 saturation of ammonium sulfate (135 gm/liter). The light precipitate formed, designated as "0.7 s.a.s. precipitate," is filtered with the aid of 2 gm of standard supercel/liter. The filtrate is discarded.

The "0.5 s.a.s. precipitate" (including the Celite) is then resuspended in 10 times its weight of water and operation 2, including the incubation at 37° C, is repeated several times until no appreciable "0.7 s.a.s. precipitate" is formed. The combined "0.7 s.a.s. precipitate" is suspended in about 10 times its weight of water and filtered with suction. The supercel residue is washed several times with water until the washing is water clear.

(3) *Fractionation with ethyl alcohol.* The combined filtrate and washings are diluted with water to a concentration of about 10 mg of protein/ml.[2] The pH of the solution is adjusted with 5 N H_2SO_4 to about 3.8, and 2 ml of saturated ammonium sulfate is added/100 ml of solution, which is cooled in an ice-salt bath to about 2° C. One-quarter of its volume of cold 95% alcohol is added

[1] The writer appreciates greatly the very helpful cooperation of Maclyn McCarty, at whose suggestion this work was initiated.

[2] The approximate concentration of protein can be determined spectrophotometrically at 280 mμ, the optical density being about 1.2/mg of protein/ml.

slowly with stirring in order to keep the temperature of the solution below 5° C. After the mixture has been stored for 24 hrs at about 5° C it is centrifuged at the same temperature. The residue is discarded, or saved for partial recovery of active material, while the clear supernatant solution is left at about −10° C for 24 hrs. It is centrifuged at the same temperature. The precipitate, called the "second alcohol precipitate," contains most of the enzymatic activity of the original "0.7 s.a.s. fractions."

(4) *Crystallization.* The "second alcohol precipitate" is dissolved in about 10 times its volume of water, after which it is brought to 0.38 saturation by addition of 60 ml of saturated ammonium sulfate/100 ml of solution.

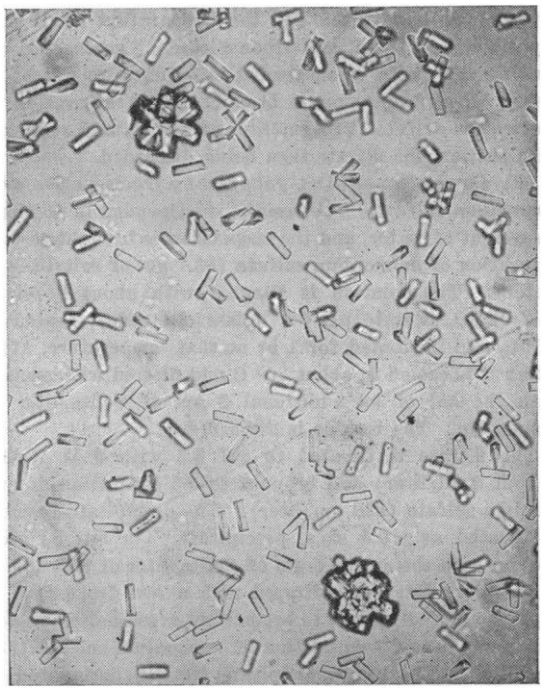

FIG. 1. Crystals of desoxyribonuclease (×234).

The precipitate formed is filtered with suction on hardened paper. It is then suspended in 3 times its weight of water and dissolved with the aid of several drops of 0.2 or 0.5 N NaOH. The solution, if turbid, is filtered clear on a small, folded Whatman No. 3 paper. The pH of the filtrate is adjusted to about 2.8 (glass electrode) with several drops of 0.2 N H_2SO_4. The solution is seeded and left at 5° C overnight and then at about 20° C for 6–8 hrs. Crystals appear at room temperature (Fig. 1).

(5) *Recrystallization.* The suspension of crystals is centrifuged. The sedimented crystals are suspended in about 3 volumes of 0.02 saturated ammonium sulfate solution and dissolved with the aid of a few drops of 0.2 N NaOH at a pH of about 4.4. The solution, if turbid, is filtered, titrated to pH 2.8, and then left at 20° C. Crystallization proceeds rapidly. The crystals, filtered with suction on hardened paper, are washed, first with ice-cold acidified 30% alcohol (1 drop 5 N H_2SO_4/100 ml), then with cold acetone, and dried at room temperature for several hours. The dry preparation is stored at about 5° C. The supernatant solution may yield more crystals on reprecipitation with ammonium sulfate; the precipitate is treated as described in step 4.

The properties of the crystalline material are now being investigated.

References

1. KUNITZ, M., and NORTHROP, J. H. *J. gen. Physiol.*, 1935, **18**, 433; 1936, **19**, 991.
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An Improved Method for Mounting Small Insects

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In the course of several years work with *Phlebotomus* sand flies we have been faced with the necessity of devising a rapid method of making permanent slide mounts of these insects which will show the delicate internal structures without shrinkage or distortion. We have tried numerous formulas of the Gater's medium type, but even when carefully ringed, they have failed uniformly in less than a year under our tropical conditions. The usual methods involving dehydration with alcohols of increasing concentration, xylol or clove oil and balsam are tedious and almost invariably result in shrinkage of the delicate spermathecae. Furthermore, treatment with alcohol and xylol tends to make the specimens very brittle. Lutz (*1*) pointed out the advantages of pure phenol as a clarifier and dehydrator, and O. Mangabeira told us of his use of it in preliminary identification of large numbers of sand flies. After a number of trials we have devised the following procedure, which has given excellent results with *Phlebotomus*, at least and should be adaptable to other small and delicate insects:

(1) Treat with strong KOH (10–20%). The specimens may be very briefly boiled, heated in a water bath, or left for several hours in the cold solution, the point being to macerate thoroughly and remove all tissue, leaving only the sclerotized structures. We find it best to remove the wings and separate the head and abdomen from the thorax before treatment with KOH. The wings are not treated with KOH but placed in the stain, where they await the rest of the specimen. Material that has been preserved for some years in alcohol is resistant to treatment with KOH, needs more prolonged treatment than do freshly caught specimens, and never makes as satisfactory mounts.

(2) Rinse in water. We find that prolonged washing or acidification is unnecessary, but all strands and bits of tissue must be removed from the specimen, as they take the stain very heavily and shrink badly in the final resinous medium, obscuring and distorting the other structures. We do the rinsing in a hollow ground slide under a binocular microscope, where the specimen may be rolled about and squeezed, the elasticity of the integument al-