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.

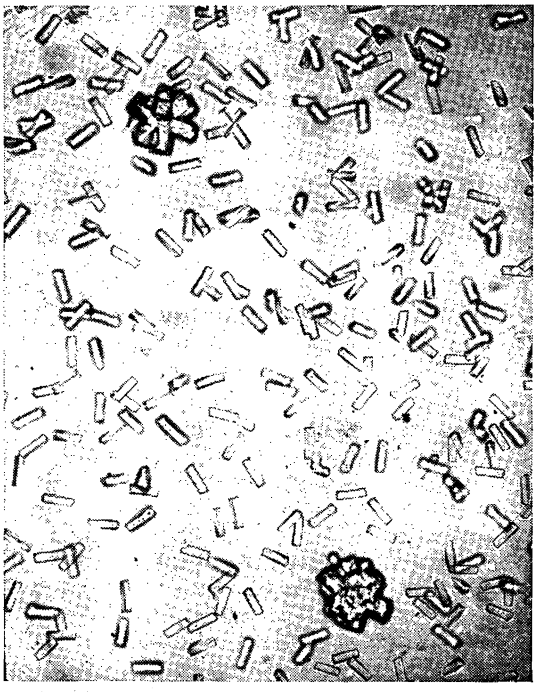

FIG. 1. Crystals of desoxyribonuclease (×234).

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.
2. MCCARTY, M. *J. gen. Physiol.*, 1946, **29**, 123.

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-

lowing the head and abdomen to be "pumped" like a rubber bulb, thus expelling any free bits of tissue within.

(3) Place in stain. The stain is made by dissolving acid fuchsin in pure phenol. Acid fuchsin is only sparingly soluble in phenol, so that a saturated solution is clear dark pink. For use, dilute the concentrated stain with at least an equal quantity of phenol in a hollow ground slide and stain for about 20 min. Should the specimen be overstained, destaining may be done in phenol to which a little KOH has been added.

(4) Rinse off the superfluous stain in pure phenol. The specimens may be left in pure phenol at least several hours without destaining, though too prolonged a stay in phenol will destain. The phenol used must be fresh and clear. We allow phenol crystals to liquefy in an open dish, after which they are kept covered. Old phenol will not make a satisfactory stain, and stained specimens will destain rapidly when placed in it.

(5) Mount. Since xylol balsam has a tendency to take up water under tropical conditions and hardens too rapidly, we use balsam or copal dissolved in pure phenol. The Canada balsam-phenol mixture darkens rather rapidly and must be made up fresh every month or so, but copal darkens much less rapidly and is equally satisfactory in other respects. In mounting, the separated head, thorax, abdomen, and wings are placed in separate small drops of phenol-copal on a small cover glass, the medium being allowed to harden a few hours or overnight. The cover slip is then turned over onto a generous drop of phenol-copal or ordinary thick balsam. This insures the specimens being close to the cover slip and accessible to the oil-immersion lens. It is important that the cover slip be supported in the final mount by chips of cover slip at the corners, as shrinkage during drying will crush and distort the specimen. The final mounts must be thoroughly dried with gentle heat; otherwise, the residual phenol may crystallize out in fine needles and spoil the specimen.

In mounting the abdomens of female *Phlebotomus*, we find it necessary to proceed gradually from pure phenol to the resinous mounting medium; otherwise, the thin-walled spermathecae will collapse. This may be done by placing the abdomen under a cover slip in phenol, either supported on a flat slide or in a hollow ground slide and gradually adding thinned resinous mounting medium at one edge, withdrawing the phenol a little at a time from the opposite side with a bit of filter paper. When the medium under the cover slip has become sufficiently concentrated, the abdomen may be transferred to the inverted cover slip in a drop of medium and allowed to dry with the rest of the specimen. In processing unidentified female specimens of *Phlebotomus*, it is wise to examine the spermathecae either in phenol or in water after KOH treatment, as the spermathecae and their ducts are best seen at this stage. If the specimen is of special value, drawings should be made at this time, since, even with the most painstaking care, shrinkage and collapse may occur in mounting.

Reference

1. LUTZ, AD. *A Folha Medica* (Rio de Janeiro), 1920, Anno 1, No. 3, pp. 1–8.

Book Reviews

Plants and environment: a textbook of plant autecology. R. F. Daubenmire. New York: John Wiley; London: Chapman & Hall, 1947. Pp. xii + 424. (Illustrated.) $4.50.

Prof. Daubenmire believes that course work in ecology should be preceded by the study of plant morphology, taxonomy, physiology, and chemistry. He also believes that the study of vegetation should not be undertaken until the student has a grasp of the numerous and often complex influences which affect the individual plant, and of the attendant responses of plant life to such influences. Thus, his approach is analytical and deductive.

For the purpose of exposition he breaks down environment into 7 "factors"—soil, water, temperature, light, atmospheric and biotic factors, and fire—and devotes a generous chapter to each. The two concluding chapters deal, respectively, with "The Environmental Complex" and "Ecologic Adaptation and Evolution." A list of 612 references in English and a detailed index are appended.

Throughout the book there is a beautiful consistency of style and organization. Both are lucid, logical, economical, and yet thorough. The illustrations, which are well chosen, are in large part original and bespeak the author's long discipline in the field. These merits, together with the sound critical scholarship which the book displays, reflect honor both upon the author and upon the fine American ecological teaching tradition of which he is a product. Happily, too, *Plants and environment* tends to round out, rather than displace in any measure, the small but vigorous teaching literature on ecology which is available to students in this country.

By the canons of criticism, if an author accomplishes what he sets out to do, and does it well, there is nothing more to say. Yet I do not believe the book would have suffered in any respect if Prof. Daubenmire had given some play to his "conception of the synthetic nature" of ecology and to his belief in the fundamental importance of the "holistic outlook." He does himself less than justice when he calls the environment the *sum* of all its factors. He knows, and later makes clear, that the relationship is not merely additive.

In short, the ultimate business of all ecology is the inseparable relationship between process and form. It seems scarcely sufficient to mention the application of Le Chatelier's theorem. The fundamental physical con-