northern South America, as far as Bolivia and Venezuela, while one isolated species (*platensis*) of the otherwise Californian genus *Ensatina* occurs in La Plata. Two species of *Hydromantes* in Italy and Sardinia constitute the only known Old World species, these being considered cogeneric with a species (*H. platycephala*) of the central Sierra Nevada.

The introduction (pp. 1–57) is an extensive discussion of general problems relating to the group.

For each species a complete chronological synonymy is given, including not only taxonomic, distributional and life history items but many references on embryology and morphology as well. In turn follow the type, type locality, range, diagnosis, formal description, comments on variation, measurements and habits, and often a paragraph discussing the relationships of the form under consideration. Habits are quoted *in extenso,* from all available sources, a feature to be strongly commended as making fairly complete accounts available to isolated students. A detailed list of localities and a map showing the range closes each chapter.

Espada's plate of *Ensatina platensis* (An. Soc. espanola Hist. Nat. Vol. IV) is reproduced as a convenience to other workers in the group.

There are few taxonomic novelties, as most of the changes in names or generic affiliation have already been announced in shorter papers by the author, and the essence of the grouping here presented has already appeared in Stejneger and Barbour's "Checklist." *Oedipus gadovii* Dunn (p. 437) from Xomelta on Mt. Orizaba is the only new form described. The author has studied at first hand all but one of the species, handling in all more than 12,600 specimens. The monograph represents ten years of labor upon a group in which relationships were by no means clear.

The only criticisms which might be levied against the book relate to certain mechanical features. A full bibliography would have been very helpful; searching for obscure references from a synonymy is not altogether easy. The table of contents relates only to genera and there is no index, in consequence of which the reader must hunt laboriously page by page for certain items. Only two unusual species are figured; outline or colored figures of at least generic types would have added greatly to the usefulness of the work. But dismissing these details, the reader finds excellent typography, a pleasing style and a wealth of interesting material. The book will long serve as the standard work of reference on its subject. Dr. Dunn and Smith College are to be congratulated on a worthy contribution.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### A TECHNIQUE FOR ARTIFICIAL FEEDING OF SANDFLIES (PHLEBOTOMUS) AND MOSQUITOES[1]

IN the course of experiments carried on by the Kala Azar Field Studies Unit,[2] Peking Union Medical College, in the attempt to transmit kala azar by means of various species of Phlebotomus, it was found that one species, *P. sergenti,* only rarely developed the flagellates of Leishmania following natural feeds on infected animals. In attempts to overcome this difficulty advantage was taken of the fact that sandflies may drink liquids offered them (Waterston,[3] 1922) and a technique was devised for feeding them rich suspensions of Leishmania.

The apparatus consists of a short length of glass tubing, in one end of which is a cork vise for holding the sandfly. In the other end is a snugly fitting cork sphere drilled to hold the feeding pipette. This permits easy adjustment of the pipette in applying it to the insect's proboscis. The cork vise and pipette holder may be turned from ordinary cork stoppers. Our apparatus was made with the aid of lathe, tool-rest and chisel improvised, respectively, from a small electric motor, the arm of a dissecting microscope and a 45-degree scalpel. Both vise and holder are turned to fit individual pieces of glass tubing. The sharpened ends of a strip of sheet brass bent double are thrust into the newly turned cork and fastened as shown. The cork is then cut longitudinally with a razor, forming the two jaws of the vise. The vise, which is kept open by the spring when free, is held securely closed when thrust into the glass tube. The vise end of the tube should be flamed, but the other end should be left unflamed, as any constriction would compress the pipette holder, which must fit tightly when in position with the tube.

Success in feeding sandflies is dependent on having the tip of the pipette properly made. It is essential that the opening be just large enough to admit the piercing stylets but small enough to exclude the labium, which is pushed back like a sleeve by the pipette. Each of the three Chinese species of Phlebotomus (*P. sergenti, P. major* var. *chinensis,* and *P. perturbans*) requires a different size of opening, within rather narrow limits for each. The

[1] Aided by grants from the China Medical Board of the Rockefeller Foundation.

[2] Charles W. Young, M.D., in charge.

[3] Annals Trop. Med. and Parasit., Vol. XVI, p. 69. 1922.

pipettes are made from pieces of capillary glass tubing of about 0.5 mm bore. The feeding end is flamed in a micro-burner until the opening is of the proper size, as determined in the first instance by actual trial on the insect, and later by comparing under a dissecting binocular with a tested pipette. The entrance canal should be short enough to permit the stylets to penetrate into the lumen of the pipette itself where they are bathed by a relatively large quantity of liquid, an important feature in connection with blood or serum suspensions, the surface of which dries quickly.

The procedure is as follows: The sandfly is etherized slightly and picked up by a leg with fine forceps. Using the cork vise with its spring as forceps, the wings are gently pressed together over the back, with the body close to and parallel with the surface of the cork. The vise with the immobilized insect, which quickly recovers from the anesthetic, is thrust into the glass tube, where it is securely held without further adjustment. Under a dissecting binocular the tip of the previously sterilized pipette is slipped over the stylets of the mouth parts, the labium folding at its base as the pipette pushes it back. This position simulates that assumed by the mouth-parts when the stylets pierce the skin in feeding naturally. The pipette is then manipulated so that the tip, with the stylets enclosed, carries the proboscis anteriorly, resulting in a bend at its base, thus accentuating the slight angle the proboscis normally makes with the head. This latter position was found to be essential for rapid feeding.

The free end of the pipette is then sterilized in the flame and a small drop of the suspension is allowed to run in. By means of another pipette fitted with rubber tube and mouth-piece this liquid is blown to the farther end, where it immediately surrounds the insect's stylets. Feeding usually begins at once and is completed within several minutes, at times within thirty seconds. It is necessary to use a fresh pipette for each insect as the minute opening is sealed by the drying of the suspension. Used pipettes may be washed by drawing water through them by means of a water suction pump.

The suspension commonly used was made as follows: Freshly defibrinated rabbit blood was centrifuged and the serum removed. Spleen tissue from a hamster heavily infected with Leishmania was ground up in this serum. A volume of spleen-serum suspension equal to that of the serum originally removed was then added to the rabbit corpuscles. Saline suspensions may also be used.

During the summer of 1926 over six hundred sandflies were fed by this method. After the considerable practice acquired in developing the technique the proportion of successful feeds was very high. The sandflies are not injured, and their subsequent behavior is quite comparable with that of naturally fed sandflies. Development of the flagellates of Leishmania was demonstrated in most of the sandflies fed with blood-spleen suspensions.

A similar method with certain modifications was successful in feeding mosquitoes (Culex). The apparatus is necessarily larger. The cork vise is cut so that the wings may be seized and held in approximately their normal position folded flat one over the other. The sandfly pipette is unsuitable on account of the length and flexibility of the mosquito's proboscis. Two pipettes are used, one fitting with the other with as little play as possible. They are made by drawing out capillary tubing to the proper diameter. The outer pipette should be broken off slantwise. Tips of both are smoothed in the flame. The outer one is slipped over the entire proboscis. The latter is thus held straight and the stylets enclosed within the labium lie approximately in the central axis of the pipette. The opening of the inner pipette is just large enough to admit the stylets but not the labium. As the inner pipette is slipped over the stylets the outer one is withdrawn slightly to allow for the bending of the labium which occurs as its tip is pushed back along the stylets. The suspension to be fed is then run in and further procedure is similar to that in the case of sandflies. Feeding experiments with mosquitoes were much less extensive than with sandflies, but served to demonstrate the feasibility of the technique.

This method could doubtless be adapted for feeding a variety of sucking insects in cases where other methods of artificial feeding, such as by means of membranes, fresh animal skin, etc., are uncertain or impracticable.[4]

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## SPECIAL ARTICLES

### EFFICIENCY OF PINHOLE PROBES[1]

BY far the most efficient probe thus far has been the conical quill glass cone, yielding as much as $s = 600$ ($s$ roughly in $10^{-6}$ atom) acoustic pressure

[4] Waterston, J. A. "A Contribution to the Knowledge of the Bionomics of Sand-flies," Ann. Trop. Med. and Parasit., Vol. 16, p. 69. 1922.

[1] Advance Note from a Report to the Carnegie Institution of Washington, D. C.