

pictus and *Mansonia perturbans*) no development of the microfilariae took place and three days after a blood meal all microfilariae appeared to be digested. Similar results were obtained with the bedbug, *Cimex*

lectularius. Three species of sandflies (*Culicoides*) showed no interest in feeding on the cotton rats.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A RAPID STAINING METHOD FOR RICKETTSIA ORIENTALIS¹

THE difficulties encountered in staining *Rickettsia orientalis*, the causative agent of Tsutsugamushi disease (scrub typhus or mite-borne typhus fever), in smears from infected yolk sac membranes and animal tissues are well known. In smears stained by Machiavello's method² or with 1 per cent. methylene blue the rickettsiae are stained deeply enough to be recognized, but the lack of contrast with the tissue background makes differentiation of the organisms difficult.

Recently Syverton and Thomas³ reported that preliminary treatment of smears with lipid solvents followed by Giemsa stain gave uniformly satisfactory results in the staining of *R. orientalis*. However, their technique is somewhat more time-consuming than the method, used in this laboratory for the past eight months, described below.

In 1944, Dr. T. J. Kurotchkin⁴ of this laboratory used a dilute aqueous solution containing both methylene blue and basic fuchsin to good advantage in staining *Rickettsia prowazeki* and *Rickettsia mooseri*, the causative agents of louse-borne (epidemic) typhus and murine (endemic) typhus respectively. In yolk sac smears thus treated the rickettsiae stained blue while the tissue background assumed a pinkish-purple color.

The present authors found the dilute stain solution to give nearly identical results with smears of *R. orientalis*, providing the smears were first treated with a lipid solvent such as xylol, ether or chloroform, to remove excess fatty substances. Xylol was found to be most convenient to use since it is less volatile and is readily kept in a glass-stoppered dropping bottle.

The method of staining is as follows: Smears of infected yolk sac membranes or other tissues are prepared, air dried and fixed by heat. The slide is flooded with xylol, drained and after drying in a current of air is immersed for five minutes in a distilled water solution containing methylene blue 1:5,000 and basic fuchsin 1:5,000. The preparation is then washed in tap water, dried and examined.

The dilute stain is readily made from 1 per cent.

¹ From the Division of Virus and Rickettsial Research, Lederle Laboratories, Inc., Pearl River, N. Y.

² H. Zinsser and S. Bayne-Jones, "A Textbook of Bacteriology," 8th edition. pp. 654-655. New York: D. Appleton-Century Company, 1939.

³ J. T. Syverton and L. Thomas, *Proc. Soc. Exp. Biol. and Med.*, 59: 87-89, 1945.

⁴ Timothy J. Kurotchkin, personal communication.

stock solutions of each of the two dyes and should be prepared daily to obtain best results.

SUMMARY

A rapid method for staining *R. orientalis* is described. Smears of infected tissues are defatted and stained in a dilute aqueous solution of methylene blue and basic fuchsin. Smears thus stained show *R. orientalis* as blue organisms on a pinkish-purple background.

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A METHOD OF OPENING VACUUM DESICCATORS

WHEN analytical samples are dried in vacuum desiccators, the covers often "freeze" and it becomes almost impossible to remove them manually, particularly when certain high-vacuum type greases are used. In some cases it has been necessary to resort to the dangerous practise of putting air under pressure into the desiccator, generally with loss of the sample being dried.

A simple and successful method, which is new to us, for opening such desiccators has been used in this laboratory for some months. A single-edged and sharp razor blade, such as the "Gem," which has a metal backing strip, is placed between the edges of the top and body of the desiccator and gently tapped with a small block of wood. The extremely thin wedge thus attained forces the lid off. Even under full vacuum, lids may be removed; thus the method is useful for removing the lids of ordinary desiccators when a vacuum is inadvertently obtained by allowing hot samples to cool therein with the lid on. Thin double-edged blades are not very satisfactory, since they are too flexible for use.

The precaution of wearing goggles should be observed to avoid possibility of injury by a flying particle of glass or steel. Careful cleaning of the ground glass surface before the cover is replaced is necessary to remove particles which might lodge in the grease and cause injury to the surface.

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