14991

A Method for Staining *Rickettsia orientalis* in Yolk Sac and Other Smear Preparations.*

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Ordinary dyes color rickettsiæ so faintly that special technics are employed to stain these microörganisms and to differentiate them from tissue elements. Satisfactory staining of rickettsiæ of endemic and epidemic typhus fever may be accomplished by means of a number of different staining technics,[1] including the Macchiavello, Castaneda, and Giemsa methods. In contrast, however, when these same techniques are applied to *Rickettsia orientalis,* the causative agent of scrub typhus fever (tsutsugamushi disease, mite-borne typhus), this microörganism stains poorly, if at all, in yolk-sac preparations. For this reason it is often difficult to distinguish suspected rickettsiæ from yolk granules and tissue particles in the background.

Because of the need for a reliable method of staining *R. orientalis* in yolk-sac smears, a study of staining technics and fixatives applied to this rickettsia was undertaken. It appeared possible that preliminary treatment of smears of infected yolk-sacs with lipid solvents might enhance the permeability of the microörganism to dyes. Accordingly, mixtures of chloroform and absolute alcohol, or of chloroform, absolute alcohol, and acetic acid, were employed for the preliminary treatment of smears, which were then stained by Giemsa's method. Such treatment was found to yield satisfactory results, and the details of the procedure are the subject of the present report.

Materials and Method. Fixative. Chloroform and absolute ethyl alcohol, with or without acetic acid, can be used in any one of the following proportions: (a) 1 part chloroform and 2 parts absolute ethyl alcohol, (b) 2 parts chloroform, 2 parts absolute ethyl alcohol, and 1 part glacial acetic acid, or (c) 3 parts chloroform, 6 parts absolute ethyl alcohol, and 1 part glacial acetic acid. The latter mixture, which constitutes Carnoy's fixative,[2] was found most satisfactory for routine use.

Stain. Giemsa stain is prepared in the following manner. To 39 ml distilled water are added 4 drops of 0.5% sodium bicarbonate and 1 ml of concentrated Giemsa stain (Grübler).

Procedure. Yolk-sac smears are prepared in the usual way and allowed to dry thoroughly. Cleaner preparations result when the yolk-sac is washed in 0.85% NaCl. The slide is then immersed in fixative for 15-30 minutes after which it is dried in a current of air, and placed in Giemsa stain for 15-30 minutes. It is then removed, dried and examined.

Results. When the foregoing method of staining was employed with smears of infected yolk-sacs, innumerable small, bluish-purple, well-defined bodies were seen which had the characteristic bipolar form of *R. orientalis* (Plate 1). These were usually extracellular, but a few densely packed intracellular rickettsiæ were also seen in some preparations. In smears of the same material which were not treated with lipid solvents before staining, only a few rickettsiæ could be seen, and these were neither sharply stained nor easily distinguishable from the background.

Smears of peritoneal fluid from mice or hamsters infected with *R. orientalis* were stained by this procedure. Well-defined rickettsiæ were seen intra- and extra-cellularly, in

* The Bureau of Medicine and Surgery of the United States Navy does not necessarily undertake to endorse views or opinions which are expressed in this paper.

1 Zinsser, H., and Bayne-Jones, S., *A Textbook of Bacteriology,* 8th Edition, D. Appleton-Century Co., N.Y., 1939, pp. 654-5, 859.

2 Mallory, F. B., *Pathological Technique,* W. B. Saunders Co., Philadelphia, 1938, p. 38.

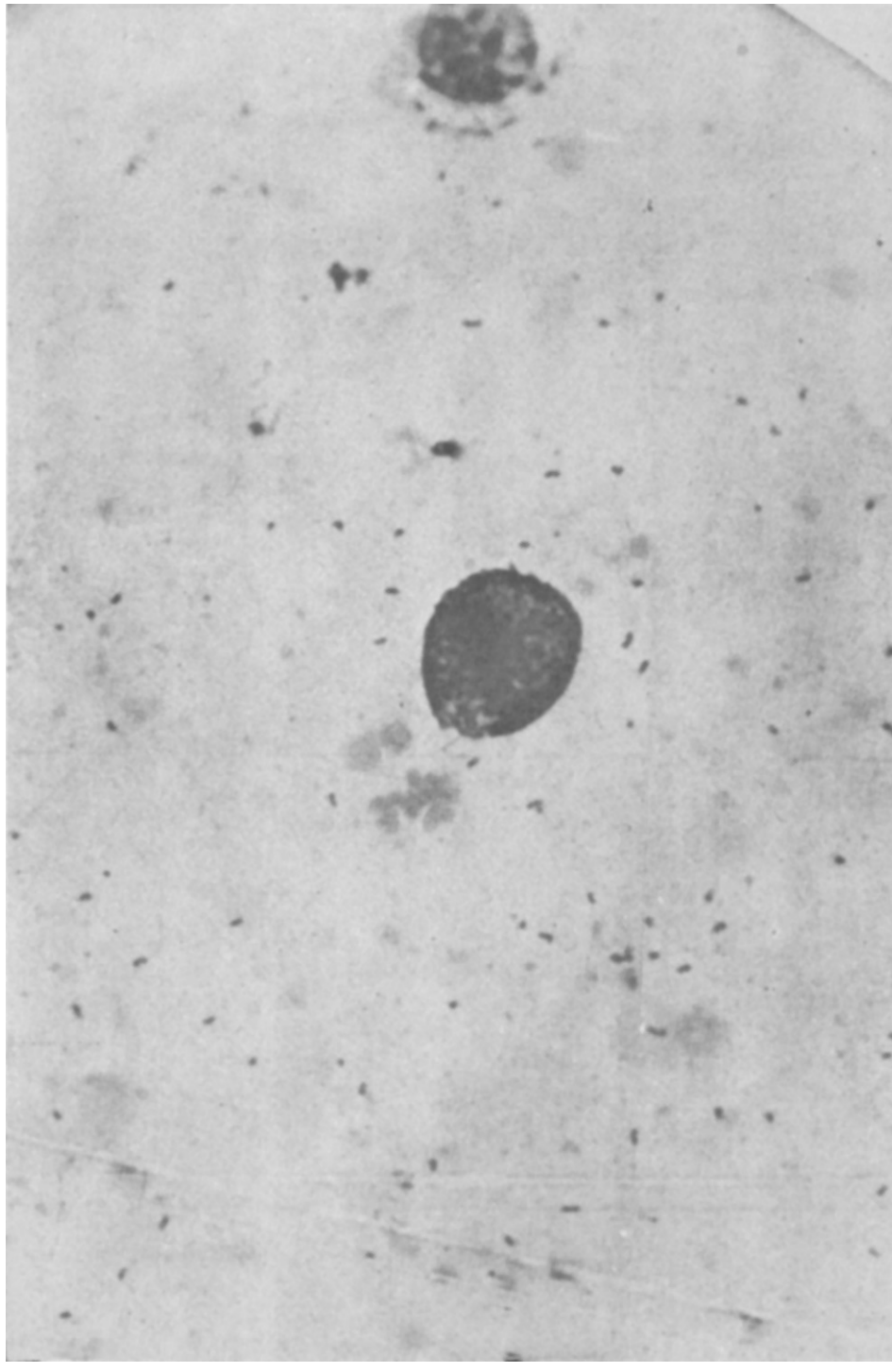

PLATE 1.
R. orientalis in smear of infected yolk-sac. Giemsa stain. × 2000.

larger numbers than in untreated preparations stained by the Giemsa method.

Summary. A method for staining *R. orientalis* in yolk-sac smears is described which yields uniformly satisfactory results. The efficacy of the method apparently depends upon the preliminary treatment of smears of infected tissue with lipid solvents.

14992

Dental Caries in the Cotton Rat. IV. Inhibitory Effect of Fluorine Additions to the Ration.*

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Miller[1] and several other workers have observed the inhibitory effect of fluorine upon the type of dental caries induced in the white rat by a coarse particle diet. Studies on the role of fluorine in the prevention of tooth decay have received increasing attention in the past few years. The cotton rat is very susceptible to the development of carious lesions when maintained on a ration containing sucrose or other soluble sugars.[2,3,4] Therefore it is fundamentally important to know the relation of fluorine in the ration to the production of this type of carious lesion.

Experimental. Fluorine as sodium fluoride (reagent grade) was added to the basal ration, 802 + 4% 1:20 liver extract, at levels ranging from 22.5 to 450 p.p.m. In these studies, the identical experimental procedure described by Schweigert *et al.*[4] was followed, with special attention to the division of litters between experimental and control groups. At the end of the 14-week experimental period after weaning, the cotton rats were sacrificed and the heads prepared for the evaluation of the incidence and extent of carious lesions. In addition to the caries evaluation, the teeth were examined for evidence of any toxic effect of the dietary fluorine.

When dextrin is substituted for all the sucrose in the basal ration, the incidence of carious lesions is greatly reduced.[2,3,4] Therefore, analyses of the dextrin and sucrose were made to determine their fluorine content. In this way it was possible to decide whether the inhibitory effect of dextrin upon the formation of carious lesions might be due to its fluorine content. The ashing of the samples was carried out in a manner similar to that described by Crutchfield.[5] The distillation was performed according to the method of Willard and Winter.[6] Determination of the amount of fluorine in the distillate was made by a colorimetric method based upon the bleaching of the alizarin-thorium lake by fluorine in a monochloracetic acid buffer.[7] Readings were made in an Evelyn colorimeter with a 520 filter.

Results. The average weekly rates of growth for the first 6 weeks and for the entire 14-week period are presented in Table I. The rate of growth is expressed as growth of males as outlined by Schweigert *et al.*[4] In most cases where 90 p.p.m. or more fluorine were

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1 Miller, B. F., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 389.

2 Shaw, J. H., Schweigert, B. S., McIntire, J. M., Elvehjem, C. A., and Phillips, P. H., *J. Nutr.*, 1944, **28**, 333.

3 Shaw, J. H., Schweigert, B. S., Elvehjem, C. A., and Phillips, P. H., *J. Dent. Res.*, 1944, **23**, 417.

4 Schweigert, B. S., Shaw, J. H., Phillips, P. H., and Elvehjem, C. A., *J. Nutr.*, in press.

5 Crutchfield, W. E., Jr., *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 57.

6 Willard, H. H., and Winter, O. B., *Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 7.

7 Unpublished data.