

## PREPARATION OF ANTIBODY TO *WOLBACHIA PIPIENTIS* OF *CULEX PAPIENS*<sup>1</sup>

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**ABSTRACT.** White mice were immunized with extracts of ovaries or testes of *Culex pipiens* either infected or not infected with *Wolbachia pipientis*. Immunoelectrophoresis of hyperimmune sera to these extracts revealed 2 antigenic components in infected gonads and 1 in

non-infected gonads. The findings suggest that 1 component is related to ovarian or testicular tissue and the other to the symbionts. Absorption of the antibody to infected ovaries by uninfected ovarian tissue removed all activity of the serum.

### INTRODUCTION

Members of the *Culex pipiens* complex contain a rickettsia-like symbiont in the gonadal cells of males and females (Hertig 1936). The symbiont, *Wolbachia pipientis*, is transmitted through the maternal cytoplasm to the offspring and can be eliminated by exposing first-instar larvae to tetracycline hydrochloride (Yen 1975). Symbiont-free (aposymbiotic) strains of *Cx. pipiens* developed by this procedure have remained free of the microorganisms in subsequent generations.

The symbionts apparently cause incompatibility because infected males are always incompatible with uninfected females; the mechanism that causes failure of embryogenesis is, however, still obscure. Yen and Barr (1973) suggest that *Wolbachia* alters sperms in such a way that they do not stimulate meiosis in uninfected eggs.

A number of attempts have been made to culture *W. pipientis* either *in vitro* or *in vivo* (Yen and Barr 1973, Wang, personal communication) as has been done with tick wolbachiae (Burgdorfer et al. 1973), but these have thus far been uniformly unsuccessful. The present study is an attempt to raise antibody against *W. pipientis* which could be used for serological identification of the organisms in cultivation studies. At present the organisms are detected and identified only by staining or

by electron microscopy, methods that lack specificity.

### MATERIALS AND METHODS

Autogenous *Cx. pipiens* of a mongrel laboratory strain infected with *W. pipientis* were made aposymbiotic by treatment with tetracycline hydrochloride. The normal and aposymbiotic strains were then used for all experiments.

Wolbachiae were detected in smears of gonads by staining with Giemsa's stain as described by Wright and Wang (1980).

**PREPARATION OF ANTIGEN.** Gonads were dissected from 3- to 5-day-old adult mosquitoes and homogenized in 1.5 ml of sterile, chilled sucrose potassium glutamate (Snyder I), pH 7, by sonication. Protein concentration (1.5-2.5 mg of protein per ml) in the homogenate was determined by the Folin-Ciocalteu method. The homogenate was stored at 4°C and was used as the immunizing and test antigen. The procedure was used to obtain:

- 1) 300 ovaries from the infected strain (1 Ov)
- 2) 350 testes from the infected strain (1 T)
- 3) 300 ovaries from the aposymbiotic strain (1 ap Ov)
- 4) 350 testes from the aposymbiotic strain (1 ap T).

**SENSITIZATION OF MICE.** Mouse immune serum was obtained by preparing mouse immune ascitic fluid; the antibody

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titers in antiserum and immune ascitic fluid are comparable (Hammon and Sather 1971).

Each homogenate was emulsified with an equal volume of Freund's Complete Adjuvant and 0.5 ml was inoculated intraperitoneally into 5-week-old female white mice. The injection was repeated twice at 12- to 14-day intervals to raise a sufficiently high antibody titer. The mice began to show abdominal distension after the second injection.

They were bled for ascitic fluid by paracentesis with an 18-gauge needle one week after the last immunization. The ascitic fluid was pooled and the fibrin clot removed by centrifugation at 2,000 rpm for 15 min. Freund's Complete Adjuvant was used as a control for immunization in one group of mice.

**IMMUNOELECTROPHORESIS.** Immunoelectrophoresis was performed in 1.75% Noble agar (Difco) with a current of 3 mA/frame for 1½ hr in a Gelman Deluxe Electrophoresis Chamber, in accordance with manufacturer's instructions. Agar was poured on microscope slides placed in a slide tray on a leveling table. [Two wells 1.5 mm in diameter and 12 mm apart were punched in the gel on each slide.] The slide tray was placed in the apparatus and the sonicated antigens in the wells. Antibodies were then added to the trough and double gel diffusion and precipitation were allowed to occur in a humidity chamber at 37°C. The slides were examined for precipitin lines 24, 48, and 72 hr later. The slides were washed in 0.85% sodium chloride solution for 24 hr to remove free proteins, dried, and stained with acid fuchsin.

## RESULTS

Figure 1a shows the immunoelectrophoretic patterns of antibody to infected ovaries (anti-1 Ov) against antigen from infected (1 Ov) and uninfected (1 ap Ov) ovaries. Antibody to infected ovaries, when tested against infected ovaries, showed a thick anodal line of precipita-

tion and a faint neutral line. The same antibody when tested against uninfected ovary antigen produced only the thick line of precipitation. When this same antibody was tested against an antigen of infected testes (Fig. 1b) only the thin precipitate line was produced. It is thought, therefore, that the thick precipitate line is the result of a reaction with ovarian antigens, and the thin neutral line of precipitation is the result of a reaction with rickettsial antigen(s).

When antibody to infected testes was reacted with infected testes (Fig. 1c), there were two precipitin lines, both rather weak. One was a neutral band and the other cathodal. When this antibody was reacted with infected ovary antigen only the neutral band was seen. The results suggest that the cathodal line is the result of reaction with testes antigen.

When antibody to infected testes was reacted with antigen from uninfected testes, only the anodal line was produced, and when this antibody was reacted with antigen from infected ovaries, only the neutral line was produced (Fig. 1d).

The presence of the faint neutral precipitin line that occurred routinely between the wells containing strain 1 gonadal antigens and strain 1 gonadal antiserum, and not between the wells with 1 ap gonadal antigens, seems to indicate that this component is related to the presence of the symbionts in strain 1 and their absence from the aposymbiotic strain. Evidently the gonads of strain 1 *Cx. pipiens* contain two antigenic components. The findings suggest that one component is related to the ovarian or testicular tissue and the other is related to the symbionts.

## DISCUSSION

Because attempts to culture *W. pipiens* have thus far been uniformly unsuccessful, a source of pure, concentrated rickettsial antigen which could be used to prepare antibody is not available. Antibody to wolbachiae prepared from ticks did not react with *W. pipiens* (Wang, personal communication). To prepare anti-

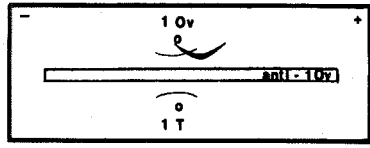
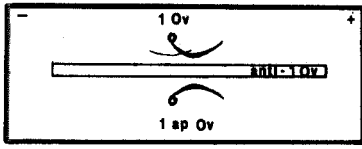


Fig. 1a and 1b. Diagrammatic representation of the immunoelectrophoretic patterns of 1 strain ovary antigen, 1 strain testes antigen, and 1 ap strain ovary antigen. The trough contains antiserum against 1 strain ovary.

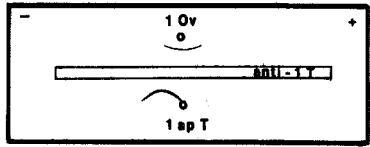
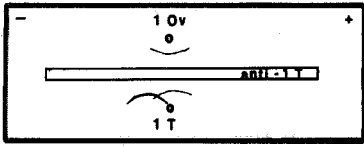


Fig. 1c. and 1d. Diagrammatic representation of the immunoelectrophoretic patterns of 1 strain ovary antigen, 1 strain testes antigen and 1 ap strain testes antigen. The trough contains antiserum against 1 strain testes.

body that could be used for the identification of *W. pipientis*, therefore, it was necessary to use infected germinal tissue of mosquitoes. Because of the small quantity of wolbachiae in mosquito gonads, it was decided to attempt to raise antibody to infected gonads and then absorb it with uninfected gonads rather than to attempt to separate rickettsial antigen from gonadal tissue.

The results indicate that antibody was raised to testes and ovary antigens and to wolbachial antigen as well. When antibody to infected ovaries was exhaustively absorbed with uninfected ovaries it lost all activity. It has therefore not been possible thus far to prepare specific antibody for the detection of *W. pipientis*. The antisera prepared could perhaps be used for the detection of wolbachiae in media lacking gonadal antigens, but the titer of the antibody to wolbachial antigen is too low to be very useful.

Immunodiffusion did not resolve the mixtures of antigens well. Although lines of precipitation could be discerned, there was considerable overlap of the lines. The ratio of antigen to antibody was varied in

attempts to increase the sensitivity of the test, but these were without success.

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## TILLAGE—A NONCHEMICAL METHOD FOR THE CONTROL OF FLOODWATER MOSQUITOES

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**ABSTRACT.** Studies were conducted both in the field and laboratory to investigate tillage as a means of controlling the production of floodwater mosquitoes, primarily *Aedes vexans*, in grassy depressions and river floodplains. Tractor plowing followed by disking produced control of mosquitoes ranging from 73 to 100 percent. This method of tillage displaced mos-

quito ova from the top 12.0 mm of the soil substrate to depths of 126 mm or more thereby trapping larvae in the soil and preventing their further development and emergence as adults. Laboratory studies confirmed that this action produced the control effect since essentially all eggs in all tests hatched, yet larval emergence decreased as soil cover over the eggs increased.

### INTRODUCTION

The concept of integrated pest management (IPM) has recently received renewed attention and emphasis as the preferred approach to pest control. Federal agencies having pest control responsibilities have been especially involved due to the recent presidential directive requiring the development and implementation of IPM in control strategies.

The Tennessee Valley Authority (TVA) since its inception in 1933 has approached mosquito control in this fashion, coordinating control activities in a truly integrated manner in which the application of pesticides is used as a supplement to the various other elements of control. This integrated approach to mosquito control is described in the 1947 manual entitled, "Malaria Control on Impounded Waters" prepared and published jointly by TVA and the United

States Public Health Service. Most of the techniques and procedures described and utilized at that time are directly applicable today. Unfortunately, however, many control planners have elected, due to financial limitations, to forego integrated pest management for the sake of initially less costly and more efficient pesticide based programs.

Integrated pest management as the basis for mosquito control on the TVA system of multipurpose reservoirs includes the application of the following preimpoundage measures: reservoir basin clearing, shoreline modifications (cut and fill), operation of dewatering units, establishment of positive drainage systems, and development of a water level management schedule compatible with mosquito control. Postimpoundage measures include: mosquito population surveillance in critical areas, water level management, drainage maintenance, mechanical control of marginal vegetation, and finally larvicidal or adulticidal application.

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