A NEW FAST FLUORESCENT DYE TECHNIQUE TO DETECT PARASITE INFECTION IN MOSQUITOES

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ABSTRACT. Many of the parasites ingested with a blood meal by mosquitoes cause midgut damage. Cellular proliferation (mitotic activity) to repair the site of injury increases RNA and DNA levels.

Squash smears were made of mosquito abdomens. Smears were agitated in the 0.01% Acridine Orange (basic orange 14) solution, pH 6.0 for 10 seconds. Smears were rinsed and mounted 1/10 M $CaCl_2$ solution.

Smears were viewed with a fluorescence microscope using a BG 12 filter. Positives were apparent with brilliant red fluorescing.

INTRODUCTION

In the course of an investigation to establish the vector status of *Dirofilaria immitis* (dog heartworm) hundreds of fleas and mosquitoes were dissected after they had fed on infected host dogs (Stueben 1954). The infected mosquitoes had midgut damage caused by the parasite migration into the body cavity. Additional experiments (Stueben 1968), revealed extensive cellular proliferation to repair these damaged areas.

In the majority of insects the epithelial cells of the midgut lack the protective cuticle, and consequently this area is often protected by a delicate peritrophic membrane. This peritrophic membrane serves to protect the midgut from damage by hard and sharp particles. De Boissezon (1930) and Huff (1934) reported that adult mosquitoes lack the peritrophic membrane because of the liquid diet and added that in the event of damage there was limited epithelial regeneration. However, Auhertot (1938) later reported that the peritrophic membrane was present in culicines and Yaguzhinskaya (1940) reported the formation of the membrane in anophelines after a blood meal. These conflicting statements raised the question of whether or not the midgut of the parasite-free mosquitoes undergoes a similar cycle.

For this study the convenient parasite model was *Dirofilaria immitis* (Leidy) the dog heartworm. The midgut of parasite-free mosquitoes showed no cellular proliferation. Parasite-infected mosquitoes had extensive cellular proliferation and a high level of mitotic activity which involves protein synthesis and in turn means that the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) levels are elevated drastically.

Bertalanffy, Masin and Masin (1956) demonstrated that fluorochroming techniques proved to be valuable in the detection and screening of cancer cells. This is because these fast reproducing malignant cells usually have nuclei with large amounts of DNA and cytoplasm with an increased amount of RNA. According to Kasten (1967) the most important use of Acridine Orange is as a fluorochrome for nucleic acids.

Acridine Orange combines with DNA and with RNA. By altering the pH level of the buffer solution it is possible to accentuate the nuclear or cytoplasmic fluorescence. The high molecular weight DNA fluoresces green and the lower molecular weight RNA fluoresces a brilliant red when the AO stain is buffered to pH 6.0. Takahoshi (1971) stressed the importance of the concentration of the dye and the pH level in order to achieve the optimum intensity of fluorescence.

METHODS AND MATERIALS

Because of the size of the cells in the midgut and method of preparation, the cytoplasmic RNA examination procedure was followed. Four study groups were set up as groups A, B, C, and D.

A. Midguts of parasite-infected *Psorophora ferox* (Humboldt) (confirmed by microdissection).

B. Midguts and abdomen of parasite infected *Ps. ferox* (confirmed by microdissection).

C. Midguts of parasite-free *Ps. ferox.*

D. Midguts and abdomen of parasite-free *Ps. ferox.*

The specimens from groups A, B, C, and D were then compressed between slides so that two smears were available per specimen.

Many staining procedures were attempted. The one that proved to be most rapid and simple was an adaptation and modification of the supravital staining technique reported by Takahoshi (1971) and Bertalanffy, Masin and Masin (1956).

The slides are placed in the 0.01% pH 6.0 Acridine Orange stain solution for 10 sec. Then the slides are removed from the stain and the excess is wiped off of the back of the slide. A 1/10 M calcium chloride solution is used as a rinse and the residual $CaCl_2$ serves as a means to mount the coverslip. The slides are then examined with a fluorescence microscope equipped with a BG 12 (blue) filter and a blue absorbing filter.

RESULTS AND DISCUSSION

Groups "A" and "B" were positive with RNA red fluorescence, when stained with the following stock solution:

1 part 0.1% Acridine Orange (basic 14)
9 parts phosphate buffer (pH 6.0) composed of: 1/15 M KH_2PO_4 (230 ml) added to: 1/15 M NaH_2PO_4 (40 ml)

On the basis of this study, a technician theoretically can:

1. Collect mosquitoes.
2. Remove abdomens of these mosquitoes.
3. Make squash smears of abdomens.
4. Stain the slides with the freshly mixed aforementioned stain preparation.
5. Have a local medical-technologist examine the smears for RNA fluorescence (using equipment normally available in the medical laboratory for "Pap" test).

This would lead to increased rapidity of screening for parasite infections such as filaria, malaria and others, where the mosquitoes are subject to midgut damage.

For the fluorescence microscopy reflected light may be more advantageous than the transmitted light because of variation in specimen densities.

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