Dyes as an Aid in the Precipitin Test for Host Blood Meals of Mosquitoes

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In 1950 Schubert and Kelly (1) described methods currently in use by CDC in Atlanta for determining the species of host blood meal in mosquitoes. Although a number of improvements over previous procedures were discussed, one defect inherent in precipitin tests in capillary tubes was not corrected at that time. This difficulty was the problem of determining quickly and accurately the interface between antigen and antiserum and the zone where the true precipitin reaction occurred. This difficulty was enhanced by the faintest cloudiness of the glass or a cloudiness in either of the reacting fluids. After some experimentation, it was found possible to resolve this difficulty by the use of dyes. The purpose of the dye was to sharpen the definition of the reaction zone and to differentiate between antigen and antiserum, thus facilitating the reading of the results.

The antiserums were colored by adding .06 ml. of a dye solution to 10 ml. of the diluted antiserums. This solution was prepared by adding 0.25 gm. of dye to 10 ml. of ethyl alcohol and diluting to 100 ml. with distilled water. The dyes tested in these concentrations were safranine O, basic fuchsin, methylene blue, crystal violet, and brilliant green. Safranine O and basic fuchsin produced a greater contrast between antigen and antiserum than did the other dyes tested. Of the two, safranine O was the most satisfactory.

Using the improved technique of Schubert and Kelley, 167 mosquito blood meals were tested with human, equine, bovine, porcine, and avian antiserums, prepared with and without dye. Of these mosquitoes, 100 were *Anopheles quadrimaculatus*, the remainder *Anopheles crucians*. These mosquitoes were from known feedings of human, equine, bovine, porcine, and avian hosts. The test gave 100 percent accuracy in detection of the blood meals of the 167 known mosquitoes. Titer and specificity of the antiserums were not altered by the addition of dye. The dye was added fresh to each lot of newly diluted antiserums.

The effects of addition of color on the precipitin tests are physical. Striking contrast results when a colorless antigen is brought in contact with the colored antiserums. The contrast between a colored fluid layered over a colorless fluid affords a simple and rapid means of determining and controlling the desired amounts of antigen and antiserums to be drawn into the capillary tubes. Using this modification, any improper layering may be easily detected. Capillary tubes sometimes are not thoroughly dried after washing; in such cases, traces of water remain in the tubes to react with one or the other of the test reagents, thereby yielding a false precipitin ring or a cloudy zone, often not at the true interface. When antiserums containing dye are employed, correct observation of the true precipitin ring at the zone of reaction between the contrasting reagents is assured. Any false reactions elsewhere than in this zone may be readily noted.

The addition of color to antiserums may prove to be of equal value in the performance of other precipitin tests, such as those used for typing streptococci, in which colorless reagents are now being employed in capillary tubes.

REFERENCE

1. Schubert, J. H., and Kelley, M. H.: The precipitin technique for determining species of host blood in mosquitoes. Modifications and improvements. J. Nat. Mal. Soc. 9(4): 341-348 (1950).

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