TABLE II. *In ovo* Neutralization of 100 Embryo $ID_{50}$ of Mouse-Brain-Propagated, Distemper Virus by Distemper Immune Serum.

| Final serum dilutions | Normal serum | Immune serum |
|---|---|---|
| 1-8 | 4/4* | 0/4 |
| 1-16 | " | " |
| 1-32 | " | " |
| 1-64 | — | 1/4 |
| 1-128 | — | " |

* No. egg membranes showing lesions/No. inoc.

in dilutions as high as 1-32 while normal serum had no observable neutralizing effect.

*Summary.* Two separate lines of the FXNO strain of egg-adapted canine distemper virus were passed intracerebrally in suckling mice 18 and 10 times, respectively. Both lines induced signs of protracted hyperkinesis. After the 7th passage central nervous system involvement became quite prominent and severe. Ataxia, muscular twitching, disequilibrium, paresis, and clonic spasms were common manifestations. The time of the first nervous disturbance decreased progressively from 8 days to 3 days in Line 1 and from 15 days to 4 days in Line 2. *In ovo* neutralization tests and ferret protection tests establish the identity of the mouse passaged agent with the original egg-adapted FXNO strain.

*Comment.* While this manuscript was in preparation, a Lederle strain of avianized distemper virus (Lederle Distemper Vaccine Mink Serial No. 6368-17A) was passaged 6 times in suckling mice with results in agreement with those reported for the FXNO strain. This would suggest the lack of strain specificity in the mouse adaptability of the avianized distemper virus.

1. Haig, D. A., *Onders. J. Vet. Sci. and An. Ind.*, 1948, v23, 149.

2. ———, *J. S. Afr. Vet. Med. Assn.*, 1949, v19, 73.

3. Cabasso, J. V., and Cox, H. R., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 246.

4. West, J. L., Thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Wisconsin, 1952.

5. Cabasso, V. J., Burkhart, R. L., and Leaming, J. D., *Vet. Med.*, 1951, v46, 167.

6. Belcher, J., Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science, University of Wisconsin, 1951.

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## Infrared Spectrophotometry as a Means for Identification of Mosquitoes.* (20526)

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In addition to the conventional taxonomic methods, various types of differentiating procedures have been employed in attempts to distinguish between closely related species of mosquitoes. The *Culex pipiens* complex in this country, for example, has been studied through cross-breeding experiments, temperature responses, susceptibility to parasitic infections as well as immunologically(1). Such investigations have been made in the search for distinguishing characteristics whereby the exact relationship between morphologically similar species might be determined. These biological tools, however, are very time-consuming and might well be supplemented by other methods. Until recently, no attempt was made to employ biochemical procedures as a means of comparing mosquito species. The first step in this direction was made by Micks and Ellis(2) in which the free amino acids of 7 species of mosquitoes were analyzed by paper chromatography. Certain quantitative differences were found between the

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various genera of mosquitoes used, while such differences between the species of a single genus were less marked.

In searching for a more precise method for comparing biochemical constituents, it was decided to use infrared spectrophotometry. The use of this method for the analysis of proteins and complex biological substances has been extended in recent years(3-11). Concomitant with this development, the potential utility of infrared spectra for the identification of naturally occurring substances has been indicated, notably among which has been the identification of bacteria(7,11). This report represents the initial studies in the application of this technic to the identification of mosquito extracts.

*Materials and methods.* The mosquitoes used were *Culex quinquefasciatus, Culex molestus* (autogenous) and *Aedes aegypti,* all of which were maintained as laboratory colonies. The larvae were fed a standard diet of finely powdered dog chow. Unfed, adult female mosquitoes were allowed to emerge in lantern chimneys and all batches were approximately the same age when used (24-48 hours). They were anesthetized with chloroform and immediately weighed, after which they were transferred to a tissue grinder and homogenized in distilled water (2 ml per 125 mg of mosquitoes). The material was centrifuged at 5000 RPM for 15 minutes and the supernatant retained. This procedure was repeated twice, resulting in a supernate free of visible suspended material. Two tenths of a milliliter of the aqueous extract was spread evenly over the surface of a silver chloride plate. The material was applied in the shape of the aperture of the liquid micro-cell which was adapted to hold a one-inch square silver chloride plate. The area of the preparation was slightly larger than the aperture so that the sample completely filled the optical path. The specimens were dried slowly at 37°C and analyzed within 24 hours after preparation. The infrared absorption spectra from 2-16 $\mu$ were determined with a Baird double-beam recording instrument equipped with a sodium chloride prism.

*Results.* It was necessary to establish the reproducibility of the method used for the preparation of the extracts. Fig. 1 presents the spectra of 4 different batches of *C. molestus* extracts. These spectra were found to be qualitatively reproducible, thus making it possible to detect the subtle differences that existed among extracts of the different species of mosquitoes studied. Although it has not been possible to reproduce with complete accuracy the quantity of absorbing material which probably accounted for the variation in the amount of light scattering, it would appear that this difficulty is also inherent in various other complex systems. This has been reported by Stevenson and Bolduan(7) with bacteria and observed by Benedict and Pollard(12) with virus preparations. Nevertheless, the quality of the films was judged by the same criteria as reported by Stevenson and Bolduan(7) namely, the use of the maximum transmission values at 5.5 and the minimum value at 6.1 $\mu$.

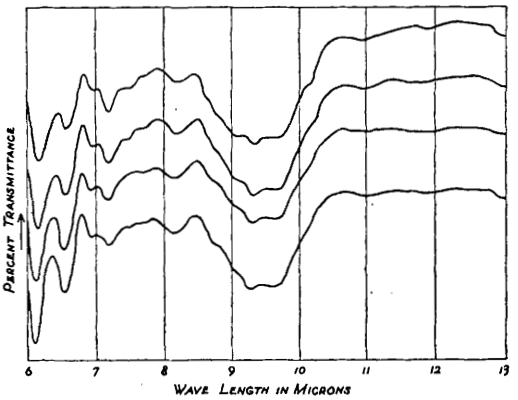


FIG. 1. Spectra of 4 different aqueous extracts of *Culex molestus* mosquitoes

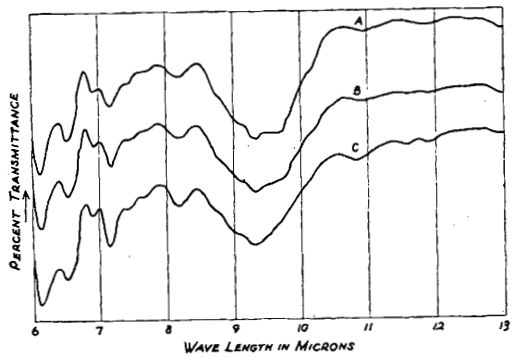


FIG. 2. Spectra of aqueous extracts of 3 mosquito species: A, *Culex molestus*; B, *Aedes aegypti* and C, *Culex quinquefasciatus.*

Fig. 2 shows the spectra of aqueous extracts of *C. molestus, C. quinquefasciatus,* and *A. aegypti* within the most informative range (6-13 $\mu$). Although the same major absorption bands were observed, sufficiently consistent differences made possible the identification of these mosquitoes. The most important differences were the relative intensities of the peaks at about 6.95 and 7.2 $\mu$, and the relative depth and shape of the broad band from about 8.6 to 10 $\mu$. In one instance, a preparation was made using *C. molestus* males and the resulting absorption bands were indistinguishable from those of the females of this species.

Six weeks storage of the smeared silver chloride plates at room temperature rendered certain changes in the spectra. There was a loss of considerable definition of the band from 8.5 to 10 $\mu$.

*Discussion.* The data given in this report indicate that infrared spectrophotometry is a simple and rapid method for the identification of the mosquito extracts studied. Moreover, sufficient differences existed among the species used to indicate that specific identification by this method is possible. It is interesting to note that the biochemical identity of mosquitoes may not follow the classical taxonomic pattern. For example, the extracts of *C. molestus* demonstrated absorption bands more similar to those of *A. aegypti* than those from a mosquito of the same genus (*C. quinquefasciatus*). It seems obvious, however, that no conclusions can be drawn at the present time regarding this point.

The characteristics of the spectra were not a function of concentration since it was observed that by titrating *C. molestus* and *C. quinquefasciatus* extracts the qualitative shapes of the curves did not change although the intensities of the bands decreased proportionately with dilution. The crude aqueous extracts were satisfactory for analysis, however, it may well be that further processing will yield preparations exhibiting even greater differences, thereby facilitating the infrared analysis. The utility of this method for the identification of other genera and species of mosquitoes is being investigated and will be the subject of another report.

*Summary.* Crude aqueous extracts of *Culex molestus, Culex quinquefasciatus,* and *Aedes aegypti* adult mosquitoes were identified by infrared spectrophotometry. The most important differences were the relative intensities of the peaks at about 6.95 and 7.2 $\mu$, and the relative depth and shape of the broad band from about 8.6 to 10 $\mu$.

1. Rozeboom, L. E., *Trans. Roy. Ent. Soc. Lond.,* 1951, v102, 343.

2. Micks, D. W., and Ellis, J. P., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 69.

3. Darmon, L. E., and Sutherland, G. B. B. M., *J. Am. Chem. Soc.,* 1947, v69, 2074.

4. Blout, E. R., and Mellors, R. C., *Science,* 1949, v110, 137.

5. Randall, H. M., Smith, D. W., Colm, A. C., and Nungester, W. J., *Am. Rev. Tuberc.,* 1951, v63, 372.

6. Schwarz, H. E., Riggs, C. G., McGrath, J., Cameron, W., Beyer, E., Bew, E., Jr., and Childs, R., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 467.

7. Stevenson, H. J. R., and Bolduan, O. E. A., *Science,* 1952, v116, 111.

8. Pollard, M., Engley, F. B., Jr., Redmond, R. F., Chinn, H. I., and Mitchell, R. B., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 10.

9. Sutherland, G. B. B. M., *Adv. in protein chemistry,* Academic Press, Inc., N. Y., 1952, v7, 291.

10. Woernley, D. L., *Cancer Res.,* 1952, v12, 516.

11. Levine, S., Stevenson, H. J. R., Chambers, L. A., and Kenner, B. A., *J. Bact.,* 1953, v65, 10.

12. Benedict, A. A., and Pollard, M., Unpublished data.

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