anti-Rh sera. Thus, it is now possible to anticipate the behavior of a post-transfusion anti-Rh serum if the Rh subtype of the donor is known, while the quality of the anti-Rh agglutinins in sera from mothers of erythroblastotic babies may be predicted by tests on the blood of the husband or infant.[17] On the other hand, the uniformity in specificity of the guinea-pig antisera is most likely due to the corresponding uniformity of the Rh-like antigens in the red cells of different rhesus monkeys.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE DEMONSTRATION OF THE PROTOZOAN PARASITE OF QUAIL MALARIA BY FLUORESCENCE MICROSCOPY

THE favorable results which have been obtained in development of diagnostic methods for detecting acid-fast bacteria by fluorescence[1] (Hagemann and others) and the results obtained by Bock and Oesterlin[2] in their studies of the action of anti-malarial drugs have suggested the potential value of this method for the diagnosis of malarial infections from blood smears. Subsequent work by the authors has demonstrated that the human parasite, *Plasmodium vivax*,[3] and the organisms of bird malaria, *P. nucleophilum*,[4] and *Haemoproteus* sp.[5] (from California Valley quail) can be stained in a satisfactory manner with fluorescent dyes.

Due to the large size of the parasite and the supply of the *Haemoproteus*, the bulk of the work in these laboratories has been done with this organism.

During the course of investigation positive staining reactions were observed with six fluorochromes. These were applied from saturated aqueous solutions to smears fixed in methyl alcohol as for Giemsa staining. The staining time is from two to five minutes. Alcoholic solutions can be substituted if the smears tend to wash off the slides with the aqueous stain. The six stains and their effectiveness in differentiating *Haemoproteus* are listed in Table 1.

TABLE 1

Stain	Nuclear color	Parasite color	Leucocyte color	Staining intensity
Beberine sulfate	Bright yellow	Golden	Yellow	+++
*Rivanol	Yellow green	Yellow green	Bright yellow	+++
Primulin	Blue	Blue white	Yellow	+++
Coriphosphine 0	Orange	Orange	Bright orange	++
Thioflavin	Yellow	Yellow	Yellow	++
Auramine 0	Yellow	Blue-white	Bright yellow	+

* 2-Ethoxy-6, 9-diamino acridine lactate.

Altering the pH of the staining medium with phosphate buffers showed a very slight increase in staining intensity in the alkaline region.

The apparatus necessary for fluorescence investigations of this nature is relatively simple. The principal innovation in equipment from ordinary microscopy is the use of a G.E. type H-4 high pressure mercury vapor lamp as the light source and a Corning filter No. 5840 which transmits the light between wavelengths of 310 mμ to 394 mμ. Ultraviolet light in this region is invisible but excites fluorescence in the fluorochromes.

The advantages of this technique if developed for the diagnosis of human malaria are many. The staining process is short, simple and reliable; the parasites, if present, stand out brilliantly as brightly fluorescent objects against a dark field; *Haemoproteus* of bird malaria and the *Plasmodia* of human malaria are readily discernible with dry lenses at magnifications not in excess of 200 ×; and the factor of eyestrain is greatly reduced.

Circumstances do not permit the full development of these techniques as applied to malarial diagnosis in this laboratory. All the results thus far obtained indicate that the method offers great possibilities in enhancing both the speed and accuracy of malarial diagnosis from blood smears. It also offers an interesting technique for the study of anti-malarial drugs (many of which are fluorescent) and their action upon the parasites.

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[17] A. S. Wiener, *Amer. Jour. Clin. Path.*, in press.

[1] P. K. H. Hagemann, *Munch. Med. Wschr.*, 85: 1066, 1938.

[2] E. Bock and M. Oesterlin, *Zbl. Bakt.*, 143: 306, 1939.

[3] Furnished by Dr. Martin D. Young and Dr. Mark F. Boyd, U. S. Public Health Service and Rockefeller Foundation, respectively.

[4] Furnished by Dr. R. D. Manwell, Syracuse University.

[5] Furnished by Dr. C. M. Herman, Division of Fish and Game, State of California.

[6] Now with the Tennessee Valley Authority, Wilson Dam, Alabama.

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