trointestinal mucosa as early as 2 hours after ingestion in guinea pigs. 3. Labeling of diverse tissues including proliferative cells of the testes was seen in rats and mice 7½ hours after ingestion. 4. Some of the H^3TDR was degraded to tritiated water while small amounts of non-volatile H^3 activity were excreted in the urine. 5. The significance of absorption of H^3TDR through intestinal mucosa is discussed as related to potential hazard.

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Received October 4, 1960. P.S.E.B.M., 1961, v106.

Staining of Malarial Parasites by the Fluorescent Antibody Technic. (26235)

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The fluorescent antibody technic has been used successfully by a number of workers to stain a variety of organisms(1, and others). The ease with which serums may be conjugated with the isothiocyanate derivative of fluoerscein and the stability of the labeled preparation has rapidly advanced the method from one of a purely research tool to one which is developing into a diganostic instrument as well. Our study was made to bring this useful and proven procedure to the study of malariology where its research and diagnostic potential appear promising. The following experiments were undertaken: (1) to determine whether natural infections produce antibodies which could be tagged with the dye to detect the organisms in the host; (2) whether antibodies could be produced in rabbits by a series of injections with malarial organisms and (3) whether one stage of the parasite, *e.g.*, the sporozoite which develops in the mosquito, could be used to produce antibodies which would react with the erythrocytic stage in the vertebrate host.

Materials and methods. Serum was collected from 2 *Macaca mulatta* monkeys which had been inoculated originally with blood containing about 2.0×10^8 parasites of *P. cynomolgi bastianellii.* Each of these animals developed a high parasitemia (average 2.0×10^5 parasites/mm^3 of blood). They apparently had a spontaneous recovery from the disease. About 7 months later both animals were reinoculated with 2.0×10^8 parasites and again developed a fairly high parasitemia (about 5.0×10^4 parasites/mm^3). After approximately 6 weeks the monkeys were inoculated for the third time with the usual inoculum of parasites. Neither animal developed a parasitemia. One of the animals was given a fourth inoculation one week later. No parasites were detected by blood smear examination for one week. At this time the animals were bled. The serums were pooled and the gamma globulin

fraction precipitated by 34% saturation with ammonium sulfate. Ammonium sulfate was removed by dialysis against 0.15 M saline buffered with 0.01 M phosphate, pH 7.1 until the dialysate gave a negative test for ammonia. The serum fraction thus obtained was labeled with fluorescein isothiocyanate by the technic essentially as described by Coons and Kaplan(2). Thin blood smears were made from monkeys infected with *P. cynomolgi bastianellii;* the smears were dried, fixed for 10 seconds in absolute methanol, and after drying were layered with the conjugated gamma-globulin fraction; after 30 minutes the labeled material was washed off with phosphate buffered saline, pH 7.1; the smears were then mounted using 10% buffered glycerol and examined by means of a Zeiss fluorescent microscope. For preparing antigen against blood stages of *P. cynomolgi bastianellii,* 3 to 5 ml of blood containing about 1.0×10^8 parasites per ml was taken from infected Rhesus monkeys; serum and the buffy coat were removed and red blood cells were hemolyzed by freezing and thawing 3 times. After each thawing the residue was washed with cold 0.15 M buffered saline. Finally, the residue was resuspended in the buffered saline and stored in the refrigerator at 4°C until used; 1 ml was injected intravenously once a week for 3 consecutive weeks into each of 2 white male New Zealand rabbits weighing about 2 kg. One week after last inoculation, the animals were bled, the gamma-globulin fraction was obtained and labeled as previously described. *Plasmodium cynomolgi bastianellii* infected mosquitoes were not available for determining whether or not antibodies against the sporozoite stage could be produced which would react with the erythrocytic stage. For this purpose, *Aedes aegypti* mosquitoes infected with *P. gallinaceum* were used. The antigenic material was prepared by grinding the heads and thoraces from approximately 150 infected mosquitoes in Hanks' basal salt solution; the sporozoite suspension thus obtained was strained through glass wool to remove large particulate matter; one ml of the suspension containing about 3.5×10^6 sporozoites was injected intravenously into white rabbits as previously described. The course of injecting the parasites, collecting, and tagging the serum was carried out as in the previous study except that fresh antigenic material was made for each inoculation.

Results. Parasites of blood smears from *P. cynomolgi bastianellii* infected monkeys showed a distinct, but not a high degree of fluorescence when stained with labeled serum from experimentally infected monkeys. The morphology of the stained parasites appeared very similar to parasites stained with giemsa by conventional means. The immunological specificity of the staining was indicated by: (1) lack of staining when slides were treated with conjugated normal serum; (2) inhibition of staining by prior exposure of the parasites to unconjugated homologous immune serum; (3) failure of the conjugated antimalarial serum to stain after being adsorbed with a suspension of the organisms and (4) failure of prior application of non-immune serum to inhibit staining. Also, the labeled immune serum failed to stain parasites of *P. gallinaceum.*

We were unable to stain the homologous parasite with the fluorescent antiserum obtained by injecting the blood phase of the parasite into rabbits.

Chicken blood smears parasitized with *P. gallinaceum* and stained with antibodies produced in rabbits with sporozoites showed a high degree of fluorescence (Fig. 1). The parasites could be seen in the red blood cells which are faintly outlined as well as is the nuclei. Specificity of staining was determined as previously described. Parasites were stained also by the indirect method of Weller and Coons(3). No staining was observed when non-immune serum was substituted for immune serum in this procedure.

The non-specific fluorescence of white blood cells was not troublesome.

Sporozoites were immobilized and agglutinated with non-labeled immune serum. Control non-immune serum appeared to have little, if any, effect on the organism. Consequently, this indicates that antibodies were produced against the sporozoites. No attempt was made to stain the sporozoites because of technical difficulties.

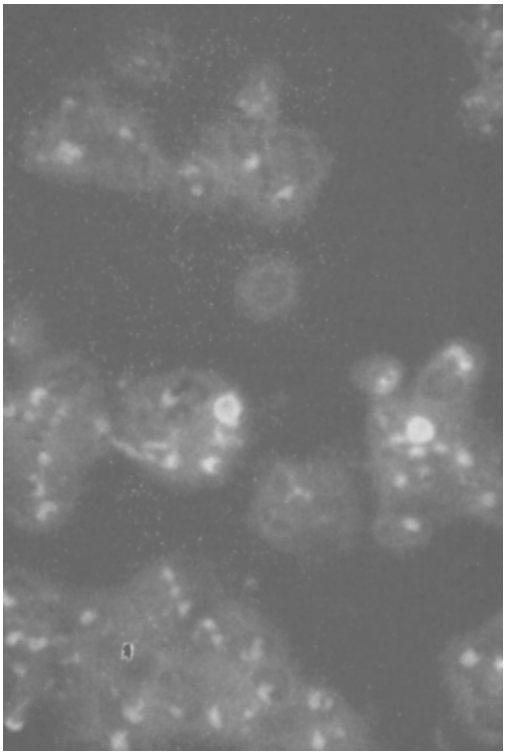

FIG. 1. Chick blood smear showing malaria parasites of *P. gallinaceum* stained with fluorescent labeled antibodies obtained from rabbits by sporozoite injections. Several non-parasitized red blood cells can be seen.

Attempts were made to stain the erythrocytic stage of *P. cynomolgi bastianellii* with the *P. gallinaceum* labeled antiserum. No staining was observed, indicating that there was some species specificity. Other species will be studied to establish the specificity of the antigens involved in the staining reaction.

Discussion. Too little is known about antigens of malarial parasites to predict much about specificity of their immunochemical reactions. One would expect that there might be some common antigens among them. However, there probably are specific antigens which would serve to distinguish them by means of immunological methods. Common antigens between species could probably be dealt with by an adsorbent technic as was demonstrated by Goldman(4) in distinguishing between *Endamoeba histolytica* and *E. coli*.

If there is immunological specificity among the various malarial organisms our finding should offer considerable encouragement in the study of the possible relationship of the monkey to the human malarial picture. Also, the demonstration that one stage of an organism can be used to prepare an antiserum to another stage of the same organism might prove useful in investigations of many parasitic diseases of man and animals in which the parasite undergoes several stages of development. One such possibility might be elucidation of the cycle of some of these organisms which are poorly understood.

Summary. Two species of malarial parasites have been stained by the fluorescent antibody technic. *P. cynomolgi bastianellii* was stained with antibodies obtained from convalescent Rhesus monkeys experimentally infected with the parasite. The erythrocytic stage of *P. gallinaceum* was stained with antibodies produced in rabbits by injections of the sporozoite stage of the organism.

The authors thank Doctors William F. Cantrell and Don E. Eyles for their help and suggestions.

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Received October 6, 1960. P.S.E.B.M., 1961, v106.