incubation period of 1 to 2 weeks required to develop polyoma (*28*) and vacuolating (*9, 22*) virus plaques.

Each of the viruses listed produces latent and chronic infections in its natural host, and each is tumorigenic. In the case of polyoma, the virus is neoplastic for species other than the mouse (*29*). Vacuolating virus has been found to produce tumors (sarcomas) when injected into newborn hamsters, with recovery of virus from the neoplasm several months after the injection (*30*). However, there have been no demonstrations as yet of tumorigenic activity in the natural primate host.

When the properties of the four agents are examined, it is apparent that they fall into a natural group for which the name, papova virus group, is proposed. The cardinal features of the viruses within the group are similarities in the size, morphology, and buoyant density of the viruses; the presence of double-stranded DNA; the absence of essential lipid; relative thermal stability; slow growth cycle characterized by multiplication within the nucleus and involvement of the nucleolus. Papova viruses produce latent and chronic infections in their natural hosts, and all are tumorigenic in their natural or other host species, or both (*31*).

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31. This work was aided by a grant from the National Foundation and grant C-4600 from the National Cancer Institute.

2 November 1961

Antibody Production in Human Malaria as Determined by the Fluorescent Antibody Technique

Abstract. No reliable serological test has been available in the past to follow the course of antibody production in malarial infections. The indirect method of immunofluorescence was utilized in this investigation to study antibody response to sporozoite-induced *Plasmodium vivax* infections in two human volunteers. Malarial antibody was demonstrated approximately 3 weeks after infection, and has persisted thus far for 121 days. These preliminary results suggest that this method of serological testing is specific and provides a sensitive means of titrating antibody produced in malarial infections.

At the present time there is no accepted serological test which is adaptable for routine use in the investigation of antibody production in malarial infections. Attempts made in the past to obtain specific complement fixation and precipitation reactions with sera from patients infected with malaria have met with only limited success (*1*). Since the fluorescent antibody technique has been used successfully in the specific staining of malaria parasites (*2*), our investigations were undertaken, utilizing this technique to follow the course of antibody production in human volunteers after sporozoite-induced infection with *Plasmodium vivax*.

Two white male volunteers, ages 40 and 41, were each bitten by 13 *Anopheles freeborni* mosquitoes heavily infected with the Venezuelan strain of *P. vivax*. Thin and thick blood smears were examined daily for parasites which were demonstrated on the 14th day after sporozoite inoculation and persisted for 25 days (patient M.C.) and 48 days (patient C.R.). Because of moderately severe clinical illness, the course of this disease was modified by intermittent antimalarial chemotherapy (Fig. 1).

Sera for antibody studies were obtained at approximately weekly intervals and frozen until ready for use. Flocculation Venereal Disease Research Laboratory tests were performed at the same intervals on all sera and remained negative throughout the course of infection. The indirect fluorescent antibody technique (*3*) was employed with thin blood films from the volunteers infected with *P. vivax* as the antigen. The blood smears were prepared when high parasite counts were present, and contained a variety of morphological forms ranging from young trophozoites to mature schizonts. A heterologous system was always used, with blood films and sera obtained from different patients. The blood smears were air dried, and stored unfixed at room temperature, 4°C, −20°C, and −50°C. The smears stored at −50°C proved most satisfactory, showing a minimum of background fluorescence after storage up to 15 weeks. Fluorescein isothiocyanate conjugated horse anti-human globulin (Sylvana Chemical Co.) was absorbed twice with rabbit liver powder, and once with rabbit bone marrow powder. Merthiolate in a dilution of 1:10,000 was added to the conjugate which was stored at 4°C until ready for use. The same lot of conjugate was used throughout, and an optimal dilution was employed to produce maximum fluorescence of the parasites with minimum fluorescence of the red blood cell ghosts. In certain preparations nonspecific fluorescence of the white blood cells occurred, but the amount was less than the specific fluorescence of the malaria parasites and did not interfere with the interpretation of the stained slides.

The blood smears were dehemoglobi-

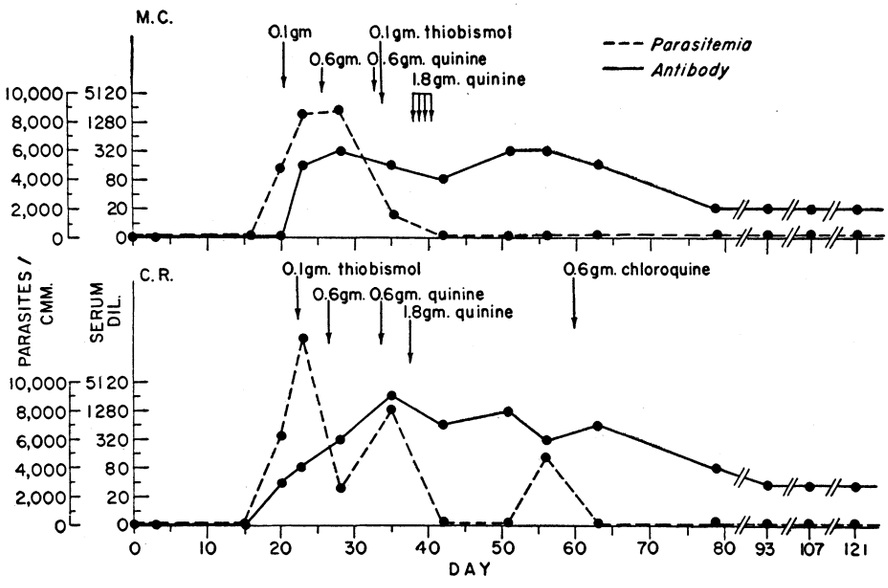

Fig. 1. Antibody production in human malaria and its relationship to parasitemia in volunteers (patients C.R. and M.C.) subjected to sporozoite-induced Venezuelan *Plasmodium vivax* infection.

nized in a Coplin jar in 0.1-percent HCl for 5 minutes, and rinsed 1 minute each in distilled water and phosphate-buffered physiological saline, *p*H 7.0. Several drops of the serum being tested were applied to the slide and allowed to react for 20 minutes at room temperature. During this period the slides were covered with a moist chamber to prevent evaporation. They were then washed for 15 minutes in buffered saline, with a change in buffer every 5 minutes. Several drops of labeled horse anti-human globulin were applied to each slide and allowed to react for 20 minutes. The slides were washed for 15 minutes in buffered saline as before, and mounted in buffered glycerine. The preparations were examined with fluorescence microscopy using wavelengths of 4100 A and 3650 A (*4*). Fluorescence was graded 1+ to 4+. A reading of 3+ or greater was considered positive. Sera from the two patients first were tested undiluted and if positive, then tested in twofold serial dilutions, beginning at 1 : 10, to determine the highest antibody titer.

The antibody levels during the course of the malaria infection in each patient are shown in Fig. 1. Patient C.R. demonstrated an antibody response on the 20th day, and patient M.C. on the 23rd day after sporozoite inoculation. Serum samples for antibody determinations were not taken at daily intervals; therefore antibody actually became apparent sometime between the 15th and 20th days in patient C.R. and the 20th and 23rd days in patient M.C. Antibody production closely followed the appearance of parasites in the peripheral circulation, and has persisted for 82 days (patient M.C.) and 59 days (patient C.R.) after the complete disappearance of parasites from the blood. The rise in titer in both patients was abrupt, reaching a high of 1:2560 on the 35th day in patient C.R. and 1:320 on the 28th day after infection in patient M.C. Although the parasitemia was affected by intermittent drug therapy, high antibody titers persisted for about 65 days after infection, when they gradually began to fall. The antibody levels in two patients were followed for 121 days and even at that time still had titers of 1:40 (patient C.R.) and 1:20 (patient M.C.).

Inhibition of immunofluorescence was accomplished by employing unlabeled and labeled horse anti-human globulin. Control sera were also tested by the indirect technique from six normal volunteers, six patients with positive Venereal Disease Research Laboratory and *Treponema pallidum* immobilization tests, one with the nephrotic syndrome, one with Sjögrens syndrome, one with disseminated lupus erythematosus, and one with active rheumatoid arthritis. There were no positive results in this group of tests save for "doubtful" results in two of the undiluted syphilitic sera giving a 2+ to 3+ reaction. However, when the latter two syphilitic sera were diluted 1:4, a negative result was obtained.

We are presently following the antibody responses in a group of human volunteers infected with the B strain of *Plasmodium cynomolgi* (*5*) in addition to further fluorescent antibody studies in patients infected with *P. vivax*. Preliminary results indicate that sera from patients infected with the B strain of *P. cynomolgi* react with *P. vivax* parasites and conversely, sera from patients infected with *P. vivax* react with B strain *P. cynomolgi* parasites, as has been demonstrated by specific immunofluorescence. This suggests some form of group reaction with regard to the antigenicity of these parasites. Other species of malaria will also be studied to determine whether common antigens are shared, and if so, what part they play in the immune response. The relationship of antibody titer to various types of antimalarial chemotherapy and to the height of parasitemia will also be under investigation. Preliminary findings appear to indicate that malarial antibody production is reflected in increased gamma globulin levels.

These studies suggest that the fluorescent antibody technique provides a specific and sensitive method for following the course of antibody production in malaria and that the technique may prove to be a useful tool in the further study of immune mechanisms in this disease (*6*).

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6. We wish to express our thanks to James V. Bennett, director of the Federal Bureau of Prisons, and to Dr. H. M. Janney, the medical director, for permission to carry out these studies. We are also indebted to Deaner Lawless and Elizabeth Guinn for their invaluable technical assistance. Lt. Col. Louis H. Muschel of the Walter Reed Army Institute of Research was kind enough to provide us with six positive syphilitic sera. The inmates who volunteered for this project certainly deserve our sincere thanks and appreciation.

11 October 1961