isolation of such a toxin was thought to be important as a rationale for the use of convalescent serum from burned patients as a therapeutic agent[1,2]. Our data do not support the thought that there is a specific antigenic toxin unique to burned tissue. It is suggested that tissue antigens of normal skin may play a role in the acute mortality observed after thermal injury. Acute mortality may be, in part, due to the fact that some normal tissue components which are usually intracellular are released into the blood upon burn injury where they act as antigens and in addition, exert a deleterious effect. The nature of these tissue components and the mechanism of their action is unknown at the present time, however antibodies formed against these components may neutralize harmful effects of these antigens and thereby reduce mortality following burns. Studies of these antigens and their toxicity are in progress.

Zusammenfassung. In Extrakten normaler und verbrannter Haut von Mäusen wurde kein Unterschied zwischen den Antigenen gefunden. Kurze Zeit nach der Verbrennung wurden Antigene auch im Serum festgestellt.

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Antigenic Changes During the Life Cycle of *Plasmodium falciparum*

Immunofluorescence (IF) studies have shown that the asexual forms of *P. falciparum* contain a cytoplasmic antigen which reacts with an antibody that is commonly present in the sera of patients who have had repeated infections with *P. falciparum* malaria[1-3]: a similar antigen is present as 'stippling' in the cytoplasm of erythrocytes infected with asexual parasites[1,4]. We now present evidence that this antigen is not detectable in the cytoplasm of immature and mature gametocytes of *P. falciparum* and thus demonstrate that there is marked antigenic difference between parasites in the asexual and sexual cycles.

The organisms were studied in the asexual cycle a) as small ring forms and intermediate trophozoites in the peripheral blood of 6 patients with acute attacks of malaria and b) as late trophozoites and schizonts, either naturally occurring in the blood of 3 infected placentas[5] or differentiated during culture in vitro[6] of peripheral blood removed from 4 patients during an acute attack of malaria. Since immature gametocytes of *P. falciparum* are only rarely found in the peripheral blood, the sexual cycle was studied in the abundant viable immature and mature gametocytes in the haemorrhagic ascitic fluid of a Gambian patient with carcinoma of the liver[7]: the findings were confirmed by study of peripheral blood containing scanty mature gametocytes from 3 children recovering from acute attacks of malaria.

The organisms were stained with the indirect IF method[8] in unfixed thin blood films with the sera of 6 Gambian adults hyperimmune to *P. falciparum* followed by fluorescein-conjugated goat anti-human-γ-globulin serum (Microbiological Associates Inc.); the sera of healthy Europeans, who had never had malaria, were used as controls. Preparations were examined with dark-ground illumination by invisible ultraviolet light either alone or in combination with visible orange light: under the latter conditions, attached dye fluoresced green and malaria pigment appeared orange. Certain stages, such as ring-form trophozoites, schizonts and mature gametocytes, were easily identified under ultraviolet illumination, but others, such as large trophozoites and immature gametocytes were distinguished by the distribution of malaria pigment (Figure 1), which was clumped in the former and dispersed in the latter[9].

At all stages of the asexual cycle, the organisms showed specific IF staining of the cytoplasm but not of the nuclei, pigment or vacuoles; there was also linear or particulate IF staining (similar to Maurer's clefts in size and distribution) in the cytoplasm of erythrocytes infected with organisms in the asexual cycle (Figure 2).

At all recognizable stages of the sexual cycle, the parasites were completely unstained. However, in the cytoplasm of erythrocytes infected with gametocytes there was widespread intense IF staining: this was confluent in the early immature gametocytes, but with late immature or mature gametocytes there was an unstained cleft, usually on the concave side of the parasite (Figure 3). It is probable that we were detecting the same antigen in the different situations, since each of 6 immune sera showed IF staining of all positive components to the same titre, irrespective of the age or stage of the *P. falciparum* parasites used as antigen. The non-immune sera never gave IF staining of erythrocytes or *P. falciparum* parasites.

Our IF observations on the antigenicity of *P. falciparum* confirm the previous reports on the asexual cycle[1,2,8], but contradict the only report on the sexual cycle, namely

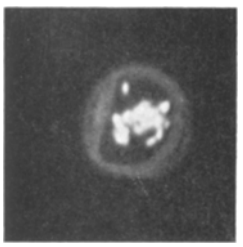

Fig. 1. Immature gametocyte of *P. falciparum* identified by the distribution of pigment using dark-ground double illumination. The orange light reflected by the pigment appears white and the fluorescent green light appears grey. × 2000.

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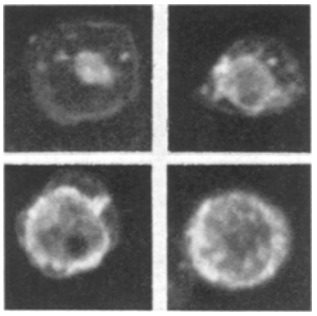

Fig. 2. Stages in the asexual cycle of *P. falciparum* from the small trophozoite (top left) to the schizont (bottom right). The cytoplasm of the parasite and 'stippling' in the cytoplasm of the infected erythrocyte are stained with the IF technique. × 2000.

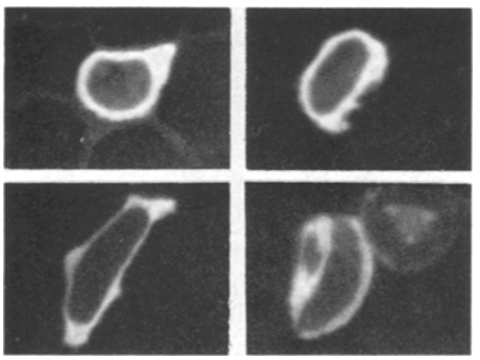

Fig. 3. Stages in the development of gametocytes of *P. falciparum* from the earlier stages (top left) to the mature gametocyte (bottom right). The parasites are unstained and the IF staining is restricted to the cytoplasm of infected erythrocytes. × 2000.

that the gametocytes stain brightly[1]. Since immature gametocytes are found in peripheral blood very rarely and then only in small numbers, we studied these forms in the parasitized haemorrhagic ascitic fluid of an unique patient[7]. The parasites were shown to be viable on in-vitro culture: furthermore the maturation of gametocytes in vivo was followed in samples of ascitic fluid removed at intervals to relieve abdominal distension[7]. It is therefore highly probable that our observations on the antigenicity of the immature gametocyte are valid, particularly since our findings in mature gametocytes in the ascitic fluid were confirmed by similar observations on scanty mature gametocytes in the peripheral blood of children convalescent from acute *P. falciparum* malaria. This antigenic difference, although previously unrecorded, is hardly surprizing in view of the other obvious changes in morphology and metabolism that the organism undergoes during gametogenesis[9]. This, or some similar antigenic difference, might explain the susceptibility of asexual parasites and the resistance of gametocytes in malarious children to passive transfer of malaria immunity by transfusion of γ globulin from hyperimmune adults[10].

The stippled IF staining of the cytoplasm of erythrocytes infected with asexual parasites has not yet been adequately explained, but it corresponds closely with the MAURER's clefts seen in ROMANOVSKY-stained preparations[4]. The cytoplasm of erythrocytes parasitized with *P. falciparum* gametocytes never contains MAURER's clefts. Single, much larger, elongated and somewhat tortuous clefts, known as 'GARNHAM's bodies' are seen on the concave side of gametocytes – these would appear to correspond to the areas lacking the IF antigen[11].

Résumé. Des études effectuées par l'immunofluorescence en utilisant les sérums de malades qui ont subi plusieurs infections de paludisme (*P. falciparum*) ont montré que l'antigène correspondant peut être détecté dans le cytoplasme de toutes les formes asexuelles du plasmodium, mais non dans les gamétocytes. Par contre, l'antigène est moins abondant dans le cytoplasme des globules rouges contenant les formes asexuelles que dans celui des cellules ayant des gamétocytes.

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The Effect of L-Asparaginase on the Synthesis and Processing of Ribosomal Precursor RNA in PHA-Stimulated Lymphocytes

The mechanism through which L-asparaginase affects growth of L-asparaginase-sensitive cells is still unclear. Amino acid incorporation into the microsomal fraction of 3 sensitive tumors is markedly inhibited[1] and this effect seems to be preceded by a pronounced inhibition of the incorporation of uridine into 18S and 28S ribosomal RNA subunits[2]. However, the relationship between the action of L-asparaginase and the decrease in ribosomal RNA synthesis awaits further explanation.

Evidence has been presented that the metabolic action of L-asparaginase is not limited to certain lymphoid tumors, but it is exerted on normal lymphoid cells as well[1,3]. Since phytohemagglutinin (PHA)-stimulated lymphocytes are characterized by a high rate of synthesis of ribosomal RNA[4], a group of experiments were performed in order to investigate the effect of L-asparaginase on macromolecular metabolism of ribosomal RNA in PHA-stimulated lymphocytes, and some of our results are here preliminarily reported.

Materials and methods. Heparinized blood from normal donors was sedimented by gravity at 37 °C in the presence of 0.6% dextran, and the supernatant, diluted with 1 volume of minimal essential medium (MEM), was filtered through a column of nylon fibers (Leukopak, Fenwal Lab.)

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