important implications. Plasmids play a significant role in conferring multiple antibiotic resistances to many pathogenic bacteria. Thus, their frequent occurrence in nature and potential for spread have had a profound impact in severely limiting our ability to adequately treat bacterial infections (*16*). For example, it is now estimated that 60 to 90 percent of all antibiotic resistances in Gram-negative bacterial pathogens and a considerable proportion of antibiotic resistances of other pathogenic bacteria are plasmid-mediated (*17*). The presence of plasmid DNA in Tp suggests that this organism also may have the potential to develop plasmid-mediated resistance (or resistances) to antibiotics as well as the capacity for some type of in vivo bacterial genetic exchange mechanism (or mechanisms), in which plasmids play a significant role (*17*). An example to reinforce this view is that of *Neisseria gonorrhoeae*, which has recently acquired plasmid-mediated resistance to penicillin from *Haemophilus influenzae* or some enteric bacterial species (*18, 19*). Our discovery that Tp has the capability of harboring plasmid DNA, which may also serve as an acceptor vehicle for transposable genetic elements, lends credibility to the warning that plasmid-mediated resistance to penicillin by Tp, either developed or acquired, may be imminent. The apparent genetic potential to acquire plasmid-linked penicillin resistance, combined with the possibility for successful "natural selection" by constant selective antibiotic therapy, raises serious concern as to how long the "wild-type" strains of Tp will continue to exhibit a relatively high degree of antibiotic sensitivity.

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Falciparum Malaria–Infected Erythrocytes Specifically Bind to Cultured Human Endothelial Cells

Abstract. *Erythrocytes infected with the late stages of the human malarial parasite* Plasmodium falciparum *became attached to a subpopulation of cultured human endothelial cells by knoblike protrusions on the surface of the infected erythrocytes. Infected erythrocytes did not bind to cultured fibroblasts; uninfected erythrocytes did not bind to either endothelial cells or fibroblasts. The results suggest a specific receptor-ligand interaction between endothelial cells and a component, or components, in the knobs of the infected erythrocytes.*

Of the four species of *Plasmodium* that cause human malaria, *P. falciparum* causes the most morbidity and mortality and presents the problem of multiple drug resistance. The clinical manifestations of malaria are caused by the asexual erythrocytic parasites which consist of a series of morphologic stages: beginning with ring forms, then trophozoites, and finally schizonts. The schizont-infected erythrocytes rupture releasing merozoites that invade uninfected erythrocytes, leading to a new asexual cycle. One characteristic of the asexual erythrocytic infection of *P. falciparum*, first noted in 1890 by Bignami and Bastianelli (*1*), is the absence of erythrocytes containing mature parasites (trophozoites and schizonts) in peripheral blood. Autopsy examination of fatal cases revealed that the trophozoite- and schizont-infected erythrocytes were found in the venules of various organs of the body (*2*).

Sequestration of infected erythrocytes within blood vessels may be of great importance to parasite survival because the parasitized erythrocytes are protected from passage through the spleen. In addition, the parasites may grow better in the hypoxic venular environment. Indeed, reduced oxygen tension has been shown to provide the best environment for parasite development in vitro (*3*). Sequestration within cerebral vessels may also play a role in obstruction of these vessels and production of the symptoms of cerebral malaria. In animal models, even when the parasitemia is low, these infected erythrocytes are retained particularly in the venules of the ventricle and atrium of the heart, adipose tissue, skeletal muscle, and submucosa of the small intestine (*4*), indicating that sequestration is not merely due to mechanical trapping of infected erythrocytes in capillaries. By light microscopy the infected erythrocytes were found to be attached to endothelial cells. Ultrastructural studies demonstrated that specialized knobs on the membrane of the infected erythrocytes in *P. falciparum* were the sites of attachment of these erythrocytes to venous endothelium (*5*).

To facilitate studies of the mechanism of adhesion of parasitized erythrocytes to endothelium, we developed an in vitro correlate of the in vivo phenomenon of sequestration. We now demonstrate that erythrocytes parasitized with *P. falciparum* bind specifically to human endothelial cells in culture and that the knobs on the infected erythrocyte membrane appear to be points of attachment to the endothelial cells.

Endothelial cells obtained from human umbilical vein (*6*) were characterized by the presence of Factor VIII antigen, typical cobblestone morphology, and Weibel-Palade bodies (see below). Erythrocytes infected with *P. falciparum* at the ring stage were obtained from the femoral vein of an infected monkey, *Aotus trivirgatus griseimembra*, and from infected humans. The erythrocytes were

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Table 1. Attachment of *P. falciparum*–infected erythrocytes to cultured human endothelial cells. In experiments 1 to 3, blood was drawn from an infected monkey and stored frozen (*7*). The blood was thawed and cultured for 34 hours to allow the parasites to mature from rings to trophozoites and schizonts. The infected blood was suspended in complete *P. falciparum* culture medium with 20 percent human AB serum. The suspension, at a hematocrit of 1 percent, was added to endothelial cells and rocked for 90 minutes at 37°C. In experiment 4, blood was drawn from the infected monkey when the parasites were at the ring stage and then cultured for 36 hours before being mixed with endothelial cells. The mixture was incubated without rocking for 90 minutes at 37°C.

Experiment	Percentage of red cells infected with trophozoites and schizonts		Percentage of endothelial cells			
	In suspension added to endothelial cells	Attached to endothelial cells	With infected red cells attached	With various numbers of attached red cells		
				1 to 2	3 to 5	10 to 100
1	1.4	100	34	29	18	53
2	2.0	93	44	8	25	67
3	2.0	97	22	45	30	25
4	3.8	97	22	17	52	31

stored at low temperatures (*7*). To test for interaction between the infected erythrocytes and the endothelial cells, we thawed (*7*) and cultured them (*8*) for 34 hours. When the parasites reached the trophozoite and schizont stages, culture medium from the endothelial cells was removed by aspiration and replaced with an equal volume of a 0.1 to 1.0 percent suspension of erythrocytes in RPMI 1640 medium containing 20 percent human AB serum. In these suspensions, 1.4 to 3.8 percent of the erythrocytes were parasitized with trophozoites and schizonts. The cell mixtures were incubated at 37°C, with gentle rocking (six cycles per minute) for 90 minutes. At the end of the incubation period, the unattached erythrocytes were removed by aspiration and the cultures were washed three times with Hanks balanced salt solution. The cells were then fixed with 2 percent buffered glutaraldehyde or absolute methanol and stained with Giemsa.

In four separate experiments, 22 to 44 percent of the endothelial cells had erythrocytes attached to their surface (Table 1). The number of bound erythrocytes ranged from a single infected erythrocyte to more than 100 erythrocytes on a single endothelial cell. Greater than 93 percent of the bound erythrocytes contained trophozoites or schizonts (Table 1 and Fig. 1a), whereas only 1.4 to 3.8 percent of the initial erythrocytes suspension placed over the endothelial cells contained these forms.

Our finding that not all the endothelial cells bound infected erythrocytes cannot be explained by the possibility that some of the cells were not truly endothelial cells, because in several experiments we simultaneously determined the presence of Factor VIII and the binding of infected erythrocytes. We observed that all cells in a dish stained for Factor VIII regardless of whether they bound infected erythrocytes or not. Transmission electron microscopy demonstrated that knoblike protrusions typical of the surface of *P. falciparum* (trophozoites and schizonts)–infected erythrocytes appeared to be the points of attachment between the erythrocytes and endothelial cells (inset in Fig. 1b). Knobs were concentrated in the area of the erythrocyte membrane in apposition with the endothelial cells (Fig. 1b). Weibel-Palade bodies known to be present in endothelial cells were observed in all cells examined.

As further evidence for cell-type specificity of attachment, the parasitized erythrocytes were layered over human dermal fibroblasts cultured under the same conditions as the endothelial cells. No erythrocytes attached to these cells. In addition, erythrocytes from normal uninfected *Aotus* monkeys and uninfected humans failed to attach to either fibroblasts or endothelial cells, indicating that the parasite-induced changes on the erythrocyte membrane were necessary for adhesion.

Two isolates of *P. falciparum* (*9*) in continuous culture in human erythrocytes were tested and were found to bind to the endothelial cells. Both isolates had knobs on the membranes of the infected red cells.

The mechanism of binding of malaria-infected erythrocytes is probably the same in vivo and in vitro. However, the general role of the knobs on the infected erythrocytes in their binding to endothelium is complex for the following reasons. In the case of *P. falciparum*, *P. coatneyi*, and *P. fragile*, the relationship appears straightforward; that is, in these malarias, erythrocytes containing mature forms of the parasites are characterized by marked sequestration along venular endothelium and have knoblike protrusions (*4, 5, 10*). Knobs are lacking on the stages of these parasites that do not adhere to endothelium, namely, the ring forms and mature gametocytes (the sexual forms) that circulate freely in the peripheral blood (*5, 10, 11*). In other species of malaria, however, knobs do not cause endothelium attachment. For example, *P. malariae*–infected red cells are covered with knobs that are morphologically indistinguishable from those of *P. falciparum*, and yet these infected cells do not sequester and continue to

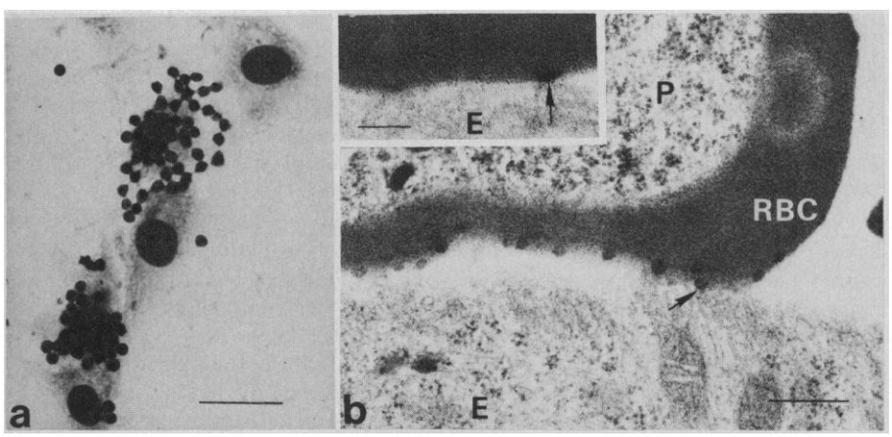

Fig. 1. (a) Light micrograph of *P. falciparum*–infected erythrocytes attached to cultured endothelial cells from human umbilical vein. Scale bar, 40 μm. (b) Electron micrograph showing a *P. falciparum*–infected erythrocyte attachment to an endothelial cell by the knoblike protrusions (arrow). Note the aggregations of knobs on the erythrocyte membrane adjacent to the endothelial cells and aggregation of cytoplasmic filaments in the endothelial cells near the attachment site. Scale bar, 0.5 μm. (Inset) High-magnification micrograph showing knobs attaching to the endothelial cells (arrow). Scale bar, 0.2 μm; *E*, endothelial cell; *P*, parasite; *RBC*, erythrocyte.

circulate in the peripheral blood (*12*). It is conceivable, therefore, that the knobs of *P. falciparum* contain several components, only one of which is required for attachment.

The knobs of *P. falciparum* were shown previously to be antigenic (*13*). Using the assay system described here we have recently identified a serum from an immune *Aotus* monkey (*14*) that abolishes attachment. It should therefore be possible to identify the adhesive component, or components, on the infected erythrocyte membrane and to investigate its immunogenic potential.

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Female Feathering in Sebright Cocks Is Due to Conversion of Testosterone to Estradiol in Skin

Abstract. *Sebright cocks develop a female feathering pattern but revert to normal male feathering after castration. Administration of testosterone to castrated cocks causes male comb development and reappearance of female feathering. Dihydrotestosterone treatment supports development of a male comb but does not induce female feathering. Since testosterone but not dihydrotestosterone is converted to estradiol in the skin of the Sebright, the female feathering appears to be the result of increased conversion of testosterone to estradiol.*

In most chickens a profound difference in plumage develops between males and females at the time of sexual maturation. In the male the feathers of the neck, cape, back, and saddle are deeply fringed because of an absence of barbules on the ends of the feather barbs, and the feathers of the tail and neck hackle are long and curved. In the female most feathers have a solid vane with less fringing, and the feathers of the tail are short and stand erect. Development of the female feathering pattern is the result of a positive effect of estrogen, whereas formation of male plumage is independent of the action of gonadal hormones (male plumage develops in males and females after castration) (*1*).

In breeds carrying the henny-feathering trait, such as the Sebright bantam and Campine, plumage is identical in the two sexes and resembles that of the females of other breeds (*2*). Castration of such chickens causes the female feathering to revert to normal male plumage (*3*), and treatment of castrated males with testosterone causes a return of the female feathering (*4*). Transplantation of the testis from the Sebright cock to the castrated Leghorn chicken results in development of a male comb but does not alter normal male feathering in the Leghorn, implying that the testis of the Sebright produces normal male hormones (*5*). Danforth and colleagues (*6*) showed that when skin is transplanted from Sebright or Campine cocks to normal males, female feathering persists in the transplanted skin whereas feathering is always of the donor type in transplants of skin from normal males to males with the henny-feathering trait. Therefore, the defect must reside in the skin itself and is apparently due to the fact that testosterone acts aberrantly as an estrogen in the skin of birds with this trait. This could occur by either of two mechanisms. Androgen could act directly as an estrogen or could be converted to estrogen in increased amounts.

The conversion of androgen to estrogen in peripheral tissues such as skin is a significant source of estrogen formation in humans (*7*), and we recently reported that estrogen formation is markedly increased in slices of skin and skin appendages (*8*) and in fibroblasts cultured from the skin of Sebright and Campine birds (*9*). Consequently, we proposed that this increased estrogen synthesis causes female feathering in males with the trait. If this thesis is correct, injecting the castrated Sebright cock with androgens that can be converted to estrogens (such as testosterone) should induce female feathering, whereas androgens that cannot be converted to estrogens (androgens with 5α-reduced A rings, such as dihydrotestosterone) should virilize the male secondary sex characteristics in-

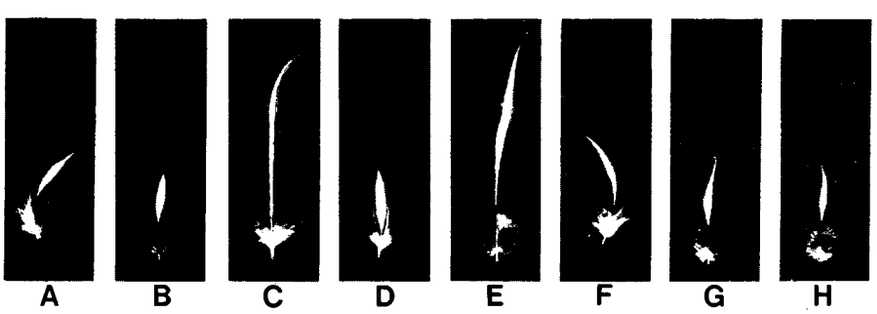

Fig. 1. Individual neck hackle feathers from Sebright bantam chickens subjected to various hormonal regimens for 3 months. (A) Intact male; (B) intact female; (C) castrated male treated with 0.1 ml of triolein per day; (D) castrated male treated with 100 μg of estradiol per day; (E) castrated male treated with 1 mg of dihydrotestosterone per day; (F) castrated male treated with 1 mg of testosterone per day; (G) castrated male treated with dihydrotestosterone (1 mg/day) plus estradiol (100 μg/day); (H) castrated male treated with testosterone (1 mg/day) plus estradiol (100 μg/day). Each dose was injected in 0.1 ml of triolein into the breast muscle.

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