channels, are smaller in mammalian cells than in insect cells or in arthropod cells in general (*15*). The present results also correlate nicely with another aspect of gap-junction particle structure. The central opening occupied by a hydrophilic stain at the intercellular end of the mammalian particles has a diameter of about 20 Å, narrowing toward the particle interior, where it was not resolved (*16*). Although this does not tell about the permeation-limiting channel diameter, it is pleasing that the value is close to the one we obtain by molecular probing.

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9. This was checked by bathing the gland cells, on the basal and luminal sides (the gland lumen was slit open for this purpose), with strongly fluorescent tracer medium for 0.5 to 1 hour and washing them; no fluorescence was detectable in the cells. (Junctional transit times were up to 2 minutes for the largest permeants.)
10. The junction-permeant tracers were incubated for 30 to 60 minutes (that is, 15 to 120 times their junctional transit times) with mashed gland cells in K medium; one would expect maximum lysosome activity in these conditions. The paper electrophoretic profiles of the tracers were the same as before incubation.
11. Lines B and RL are representatives of mammalian cell group I and 3T3-BALB/c of mammalian cell group II. Group I has somewhat less restrictive cell-to-cell channels (*3*). See (*3*) also for media and culture of the mammalian and insect cells.
12. These linear peptides' junctional permeance, nonjunctional membrane impermeance, and stability in cytoplasm have already been shown (*3*).
13. Microinjection into the salivary gland cells was hydraulic (*2*). Injection into the cell cultures was iontophoretic (*3*); the tracers, in KCl solutions, were ejected by solvent drag.
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Plasmid DNA in *Treponema pallidum* (Nichols): Potential for Antibiotic Resistance by Syphilis Bacteria

Abstract. *A plasmid DNA structure (approximate molecular weight = 7.5 × 10^6) was identified in the human pathogen* Treponema pallidum *(Nichols). The inability to isolate this plasmid from rabbit host tissue and the total lack of DNA homology of the plasmid with rabbit DNA has confirmed its* Treponema pallidum *origin. The observation documents a newly recognized and potentially significant genetic capability for* Treponema pallidum.

There is a paucity of information concerning the genetics of the bacterium *Treponema pallidum* (Tp), the etiological agent of syphilis (*1–3*). This is due to the inability to cultivate this organism in vitro (*4*) and to difficulties in the isolation and purification of Tp cells grown in rabbit testicles (*5*). We now report a plasmid DNA component in Tp (Nichols) that was identified during isolation and purification of Tp DNA for cloning studies. To the best of our knowledge, this is the first report demonstrating the presence of plasmid DNA among the *Treponema*. The observation raises concern about the possible future course and pathogenesis of syphilis, especially with regard to the potential acquisition or emergence of extrachromosomally mediated resistance to antibiotics to which wild-type strains have been so exquisitely sensitive.

The Nichols strain of Tp, which was isolated from the cerebrospinal fluid of a syphilitic patient in 1912 (*6*) but still remains pathogenic for humans (*7*), was used as the representative human pathogen for our study. Crude bulk DNA was isolated from this organism and chromatographed over Sephacryl S-300 (Fig. 1) for the routine removal of contaminating RNA fragments (*8*). The elution profile of this DNA on Sephacryl revealed at $A_{254\ nm}$ a peculiar shoulder just prior to the major RNA peak. This shoulder has not been previously observed during the chromatography of other types of prokaryotic and eukaryotic DNA samples prepared in our laboratory. Agarose gel (0.8 percent) electrophoresis of this purified material resolved a faint but sharp DNA band that migrated slightly more slowly than a 23,000-base-pair (1.5 × 10^7 dalton) marker of linear DNA (Fig. 2A, lane 3). The DNA material was isolated several times from independent preparations of purified treponemes, suggesting that a plasmid DNA component had been identified in Tp cells.

The migration of the plasmid DNA band in the agarose gel was consistent with that of many mitochondrial DNA's of eukaryotes (*9*). However, the treponemal origin of the plasmid DNA was supported by our inability to isolate a similar DNA species from testicles of sham-infected rabbits. We were also unsuccessful in purifying an analogous DNA component from approximately 3 × 10^8 rabbit testicular fibroblast tissue culture

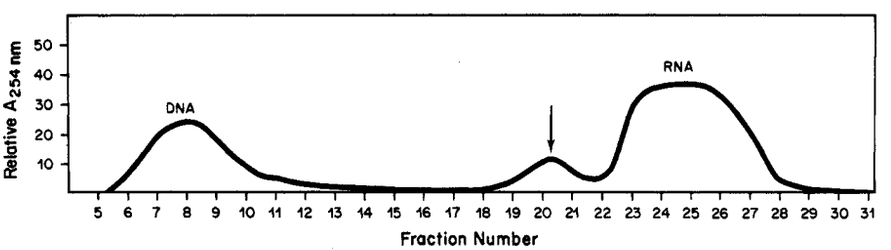

Fig. 1. Sephacryl S-300 column chromatography of crude *T. pallidum* bulk DNA. The *T. pallidum* was cultivated in the testicles of five cortisone-treated rabbits (*20*). Treponemal cells were extracted from diced testicles aerobically in 50 ml of phosphate-buffered saline (*p*H 7.2) at 23°C for 30 minutes (rotary shaking at 180 rev/min). Rabbit tissue debris was removed from the treponemal suspensions by centrifugating twice at 500*g* for 10 minutes. Treponemes were then collected by centrifugation at 16,000*g* for 30 minutes and washed twice by centrifugation in hypotonic lysis buffer (HLB) (25 m*M* tris-HCl, 10 m*M* EDTA, *p*H 8.0) to liberate and remove the DNA of the remaining intact testicular cells (*21*). Enumeration by dark-field microscopy (*22*) indicated a recovery of approximately 2 × 10^{10} intact treponemes, which were first treated with lysozyme (100 μg/ml) for 10 minutes at room temperature (*23*) in 8.5 ml of HLB and then lysed and incubated (37°C, 5 minutes) in the presence of ribonuclease A (50 μg/ml) and 0.8 percent sodium tri-isopropylnaphthalenesulfonate (*15*). The lysate was made 0.2*M* with respect to NaCl and extracted with phenol; the crude treponemal DNA was precipitated at − 20°C by the addition of two volumes of ethanol and allowed to stand overnight before collection by centrifugation at 23,500*g* for 45 minutes. The bulk DNA was dried, dissolved in 1 ml of buffer (10 m*M* tris-HCl, 10 m*M* NaCl, 1 m*M* EDTA, *p*H 7.6), and chromatographed on Sephacryl S-300 to remove RNA fragments (*8*). The arrow indicates an elution profile shoulder (*T. pallidum* plasmid DNA) not normally observed for other DNA's (*8*).

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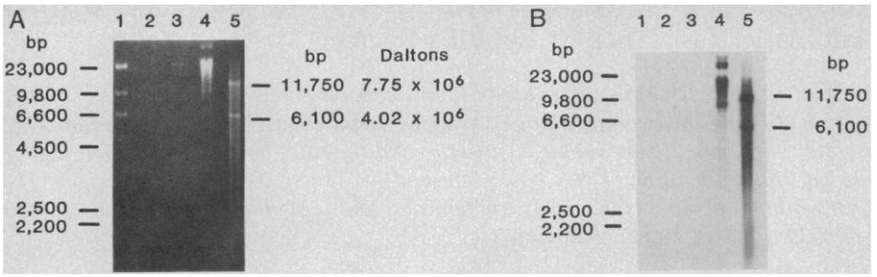

Fig. 2. Agarose gel electrophoresis and Southern transfer of λ, rabbit, and Tp plasmid DNA's. (A) Lane 1, λ-Hind III fragments. Lane 2, DNA extracted from sham-infected rabbit testicles. Lane 3, Tp plasmid DNA. Lane 4, total rabbit liver DNA enriched with mitochondrial DNA. Rabbit liver mitochondria were partially purified (*24*), and the DNA isolated consisted of about 50 percent rabbit chromosomal DNA and 50 percent mitochondrial DNA. Lane 5, total rabbit liver DNA digested with Eco RI endonuclease. Electrophoresis of an 0.8 percent agarose gel (15 cm horizontal) was conducted for 25 hours at 20 V in buffer containing 20 m*M* tris, 10 m*M* sodium acetate, 9 m*M* NaCl, 1 m*M* EDTA, ethidium bromide (1 μg/ml), *p*H 8.2. The migration of the Tp plasmid DNA band in lane 3 did not correlate with its actual size because of its open circular conformation on isolation (Fig. 3). After visualization, the DNA samples were transferred to nitrocellulose paper (*11*), and the agarose gel was again stained with ethidium bromide to ensure that all DNA samples were transferred. The DNA samples on the filter were then hybridized with ^{32}P-labeled (*12*) total rabbit DNA probe (prepared from the excess of material shown in lane 4). After autoradiography for 72 hours (B), hybridization was evident only with rabbit DNA's (lanes 4 and 5). Some hydridization with sham-infected rabbit DNA (lane 2) was barely detectable. The Tp plasmid DNA band (lane 3) showed a complete lack of DNA homology with total rabbit DNA probe.

cells (*10*). These findings indicated that the plasmid DNA component from Tp was not rabbit mitochondrial DNA.

The treponemal origin of the plasmid DNA was confirmed by the use of the Southern hydridization technique (*11*) (Fig. 2A). A portion of the rabbit DNA preparation was also digested with the restriction enzyme Eco RI (lane 5) to visualize fragments of the mitochondrial DNA. DNA isolated from the sham-infected rabbit testicles (lane 2) was not visible on the agarose gel by the ethidium bromide staining method employed, indicating that very little rabbit DNA was present in this "sham" DNA preparation. Lane 3 demonstrates the presence of the sharp plasmid band from Tp. Lane 4 shows the electrophoresis of the undigested total rabbit liver DNA preparation which contains approximately 50 percent mitochondrial DNA. The presence of the mitochondrial DNA is more evident in lane 5, in which the DNA was digested with Eco RI to reveal two major mitochondrial DNA digestion fragments among the smear of chromosomal DNA fragments. The cleavage pattern and size of the two mitochondrial DNA Eco RI fragments were consistent with the Eco RI digestion patterns of mitochondrial DNA's from other sources (*9*). When these DNA samples were transferred to nitrocellulose filters and hybridized with nick-translated (*12*) ^{32}P-labeled total rabbit DNA probe, autoradiography showed that only the rabbit DNA preparations hybridized strongly to the total rabbit DNA probe (Fig. 2B, lanes 4 and 5). A very slight smear, not in the region of the Tp plasmid DNA band, was barely detectable in lane 2 (Fig. 2B) which contained sham-infected rabbit DNA. The fact that some hybridization was detected in lane 2, where no visible DNA material was originally observable by ethidium bromide staining of the agarose gel (Fig. 2A), emphasized the great sensitivity of the Southern blotting method. Furthermore, the Tp plasmid DNA band (Fig. 2B, lane 3), which contained a DNA concentration at least an order of magnitude higher than sham-infected rabbit DNA in lane 2 (Fig. 2B), showed a complete absence of hybridization; this indicated a complete lack of DNA homology with the rabbit DNA probe. The fact that total rabbit DNA probe hybridized to both itself (Fig. 2B, lane 4) and the Eco RI–digested material (Fig. 2B, lane 5) suggested that both chromosomal and mitochondrial DNA components of the total rabbit DNA preparation were labeled equally well by the nick-translation technique. The DNA from Tp was not used as labeled probe in this approach because it is very difficult to show unequivocally that a Tp DNA preparation is totally free of contaminating rabbit DNA. Thus, although our conclusion is based on a negative result (using rabbit DNA probe) rather than a positive one (using Tp DNA probe), it appeared that the former approach would be significantly more reliable.

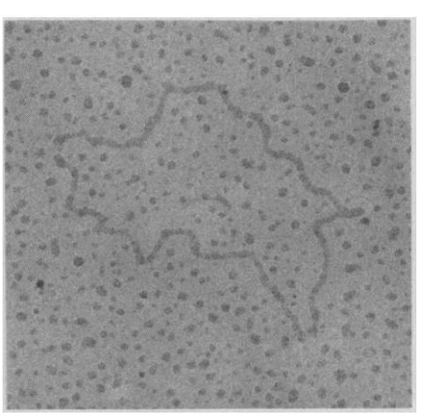

Fig. 3. Electron micrograph of an open circular plasmid DNA molecule from Tp. Average size was approximately 3.62 μm in length or 7.5×10^6 daltons (*14*). If it is assumed that the quantitative recoveries are correct, the calculated copy number is approximately ten copies per Tp cell.

DNA molecules migrate in agarose gels not only according to size but also conformation. Because the conformation of the Tp plasmid was not known, agarose gels were not useful in determining the molecular weight of the Tp plasmid DNA. Consequently, Tp plasmid DNA molecules were spread and mounted for electron microscopy (*13*), and the size of the plasmid molecules was estimated from direct visual measurement using the established relationship of 2.07×10^6 daltons per micrometer (*14*). Electron micrographic analysis revealed (Fig. 3) the circular plasmid DNA molecule to average approximately 3.62 μm in contour length corresponding to 7.5×10^6 daltons, thereby possessing genetic material for about 7 to 15 genes. No supercoiled (covalently closed circular) plasmid structures were observed by electron microscopy, but data are insufficient to permit conclusions about the "native" conformation of this plasmid DNA in Tp. However, it is unlikely that the Tp plasmid DNA represents an artifact of DNA isolation or bacteriophage DNA since circular DNA molecules do not occur as artifacts of DNA isolation, and bacteriophage DNA is not known to persist extrachromosomally inside bacterial cells.

The amount of plasmid DNA recovered from Tp bulk DNA preparations was low but not unexpected; approximately 35 μg of purified chromosomal Tp DNA and about 0.3 μg of plasmid DNA were isolated from 2×10^{10} Tp cells. Our total yield of DNA isolated per Tp cell was in agreement with previous attempts to isolate Tp DNA (*15*). The small amount of plasmid DNA recovered was also not inordinate in view of the size and complexity of the Tp genome (9.05×10^9) (*1*) compared to the low plasmid content of the Tp bacterial cell.

Despite current limitations for further structural and functional analyses of the plasmid DNA from Tp, its discovery has

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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