3B are the transmittance data as a function of off-axis angle. For the 0.75% Ni sample, the light transmittance remains nearly 90% even at an off-axis angle as much as 30° and decreases only slightly to ~85% at 45° orientation. The angle dependence is slightly more severe in the 1.5% Ni sample (which contains twice as many vertical columns as the 0.75% Ni sample), as might be anticipated from a simple geometrical line-of-sight consideration.

We evaluated the electrical conductivity of the composite medium along the vertical direction (through resistance) by four point resistance measurement, using a constant dc current of 10 mA. The composite sheet was sandwiched between two circuit boards (the lower board contained multiple 1.25-mm-wide conductor lines running in the x direction, whereas the upper board had one 1.25-mm-wide conductor line running in the y direction). A nominal pressure of 5 psi (0.035 MPa) was applied during the measurement to ensure good contacts between the metal spheres. The average through-resistance of the composite medium so measured was ~370 milliohms per 1.25 by 1.25 mm^2 contact pad area. For the given sheet thickness of ~150 μm, this resistance value translates into an apparent z-direction resistivity of ~0.39 ohm-cm for the composite material. Because there is only 0.75% by volume of the conductor present, the resistivity of the column itself is much lower (~2900 microohm-cm).

The electrical conductivity of the composite medium in the x-y direction was measured to be negligible, as might be expected from the microstructure. The in-plane resistivity was $>10^9$ ohm-cm, a value representative of the insulative polymer matrix.

An optically transparent, z-direction conductive medium such as described here may be useful as a pressure sensor for visual communication devices such as write pads or finger-touch-sensitive layers on display screens (*2*). The transparency of the pressure sensor is an advantage for tracing an underlying pattern such as a map or chart or for minimizing the loss of display quality underneath a touch-sensitive screen.

In order to make the present composite medium more suitable for transparent write pad type applications, we have further modified the material to achieve electrical on-off switchability. A very thin layer (~5 μm) of silicone elastomer was spray-coated over the top surface of the cured composite material. We have discovered that this extra layer could serve as an insulating barrier at a low applied pressure such as that exerted by a hand resting on the write pad, but it allows z-direction electrical conduction above a certain threshold pressure, for example, as exerted by a tip of a writing stylus.

The threshold pressure, which can be adjusted by controlling the barrier thickness, is in the range of 10 to 50 psi (0.069 to 0.345 MPa). Below this threshold, the through-resistance for a 1.25 by 1.25 mm^2 pad was greater than 20 megohms, whereas above the threshold the resistance was typically less than ~0.8 ohm. The underlying mechanism for this switchability is not clearly understood. However, it is most likely caused by the upper end of the vertical columns (slightly protruding as shown in Figs. 1 and 2) puncturing through the insulating barrier layer above the threshold pressure and then retracting when the pressure is reduced.

Schematically illustrated in Fig. 4 is an assembly constructed to demonstrate the feasibility of a write pad device that uses the composite medium containing ~1% by volume of the particles. The medium (15 cm by 15 cm by 150 μm) was sandwiched between two Mylar sheets (~75 μm thick) coated with ITO with the conductive side facing the composite medium.

The total electrical resistance was 4827 ohms. It includes the resistance from one edge of the upper ITO layer to the location of the stylus 6 cm away from the edge plus the z-direction through-resistance in the composite medium plus the resistance from the stylus to the same-side edge of the lower ITO layer (Fig. 4). (A normal writing pressure by the tip of a pencil is estimated to be ~100 psi, which is over the threshold value of 10 to 50 psi.) Because the through-resistance in the medium contributes not more than ~1 ohm to the combined resistance, the above resistance value represents primarily that of the ITO layers. As the location of the stylus changes, the measured resistance also varies, allowing the x-y coordinate positions to be sensed by a number of different schemes (*3, 4*). The total resistance for the case of a hand resting on the pad (~2 psi pressure, well below the threshold) was greater than 20 megohms, thus causing no interference with the position sensing.

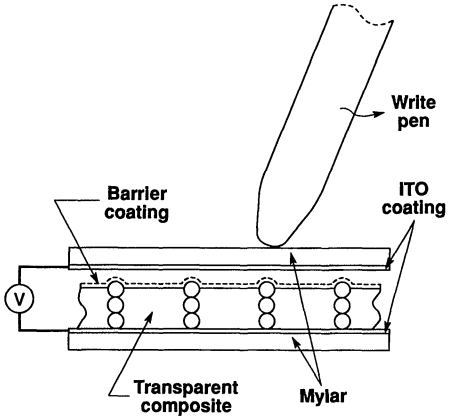

Fig. 4. Schematic illustration of an exemplary write pad assembly using the composite medium.

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Sporogonic Development of a Malaria Parasite in Vitro

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The sporogonic cycle of the avian malaria parasite *Plasmodium gallinaceum* was completed in vitro. Ookinetes (motile zygotes) were seeded onto a murine basement membrane–like gel (Matrigel) in coculture with *Drosophila melanogaster* cells (Schneider's L2). Transformation into oocysts as well as subsequent growth and differentiation were observed in parasites attached to Matrigel and depended on the presence of L2 cells. Sporozoites were first observed on day 10 in culture. Specific circumsporozoite protein antigenicity was identified in mature oocysts and in sporozoites. It is now possible to follow the entire life cycle of *Plasmodium* in vitro.

SIGNIFICANT PROGRESS HAS BEEN made in the culture of the intracellular vertebrate stages of malaria parasites. The blood stages of many species multiply readily in continuous culture (*1*), and the exoerythrocytic cycle can also be completed in appropriate cells in vitro (*2*). These achievements stand in marked contrast to the limited success in the cultivation of the

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extracellular mosquito stages of *Plasmodium* (*3*). Although ookinetes of both *P. gallinaceum* (*4*) and *P. berghei* (*5*) can be produced in culture, the development of oocysts and sporozoites is not observed in vitro (*6*).

The sporogonic development of malaria parasites in the mosquito vector comprises an elaborate sequence of stages beginning with gametocytes in the bloodmeal and culminating about 10 days later with sporozoites in the salivary glands (Fig. 1). The critical transformation from the single-celled ookinete to the mature oocyst, containing hundreds or even thousands of sporozoites, occurs in the hemocoel of the mosquito, after the parasite has come to lie between the epithelium and the basement membrane of the midgut (*7*). Laminin and collagen type IV, the major constituents of *Drosophila* basement membranes (*8*), were also identified in the basement membranes of *Aedes aegypti* and in oocyst capsules of *P. gallinaceum* developing on the midguts of the mosquitoes (*9*). This observation suggested that extracellular matrix components may be important for oocyst development in the mosquito hemocoel. Matrigel, a basement membrane–like extract derived from the murine Engelbreth-Holm-Swarm tumor, contains laminin, collagen type IV, heparan sulfate proteoglycan, entactin, and several growth factors (*10*). Matrigel promotes attachment, growth, and differentiation of a diverse array of cultured cells from a wide spectrum of animal hosts. Schneider's *D. melanogaster* line 2 (L2) cells (*11*) produce and secrete collagen type IV (*12*). In this report, we describe the conditions for culture of the mosquito stages of *P. gallinaceum* in vitro and demonstrate that a Matrigel substrate combined with L2 cells enhances the efficiency of this process.

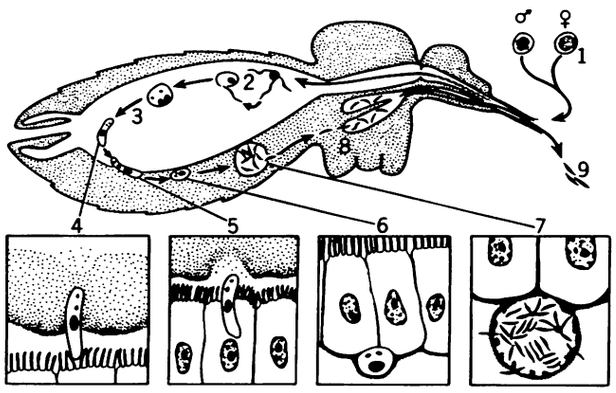

Fig. 1. The developmental sequence of *Plasmodium* in mosquitoes. Approximate times for *P. gallinaceum* developing in *Aedes aegypti* at 25° to 27°C are given in parentheses. 1, Gametocytes ingested by a female mosquito feeding on a malaria-infected vertebrate. 2, Gametes become extracellular. Male gametes fertilize female gametes to produce zygotes (15 to 60 min). 3, Zygotes transform into elongate motile ookinetes (16 to 24 hours). 4, Ookinetes penetrate and cross the peritrophic membrane (24 to 30 hours). 5, Ookinetes traverse the midgut epithelium and become lodged under the basement membrane (30 to 40 hours). 6, Transformation into oocysts (40 to 60 hours). 7, Mature sporozoites exit the oocyst (9 to 11 days). 8, Sporozoites invade the salivary glands (10 to 12 days). 9, Mosquito transmits sporozoites during bloodmeals (12 days and thereafter).

Ookinetes (*13*) and L2 cells (*14*) in a ratio of at least 1:20 were mixed, pelleted, and resuspended in oocyst-culture medium (*15*). The suspensions were pipetted on top of solidified Matrigel (*16*). Cultures were maintained in the dark at 26° ± 1°C. Flasks were kept tilted to ensure that the majority of ookinetes would settle on the Matrigel. Cultures were gassed daily with a mixture of 5% CO_2, 5% O_2, and 90% N_2. L2 cells remained in suspension and were replaced every other day when the oocyst medium was changed. Because all unattached parasites were lost when the medium was changed, development was followed only in parasites that adhered to the Matrigel.

Most ookinetes either settled directly on the Matrigel or migrated to it. We observed the transformation of ookinetes into oocysts in parasites that adhered to the surface of Matrigel and in parasites embedded in it. This was shown by the change in shape from the elongate wormlike ookinete to the rounded form of the oocyst. Approximately 50% of the attached oocysts attained a diameter of 7 μm by day 3. As they matured, oocysts either maintained a roughly spherical shape (10 to 20%) or became elongate (80 to 90%). Both elongate and spherical oocysts developed at approximately the same rate as in *Ae. aegypti* mosquitoes, and reached a diameter of 20 to 30 μm (spherical) or 15 by 40 μm (elongate) on days 5 to 7. At this stage, mitotic spindles appeared within the lobed syncytical nucleus, and distinct islands of rough endoplasmic reticulum and mitochondria were present in the cytoplasm (Fig. 2A). Oocysts were surrounded by thin, electron-dense capsules similar to those observed in vivo (*7, 17*), but more delicate (Fig. 2A).

The best results were achieved in flasks seeded with approximately 1000 ookinetes per square millimeter of Matrigel. Some 10 to 30% of the oocysts developed to maturity, reaching diameters of 35 to 50 μm (spherical)

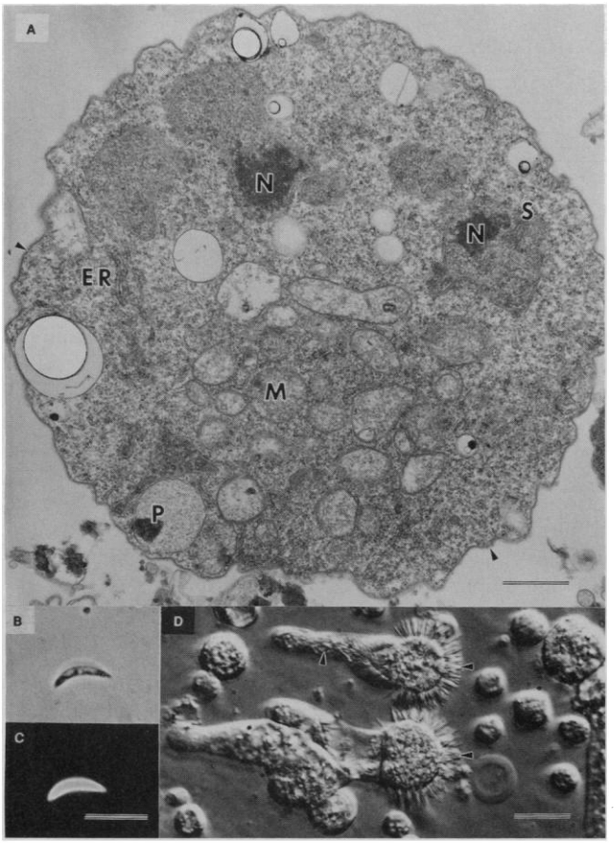

Fig. 2. In vitro development of sporozoites. (**A**) Electron micrograph of a 5-day oocyst. Nuclear lobes containing nucleoli (N), endoplasmic reticulum (ER), mitochondria (M), mitotic spindle (S), and pigment granule (P). Arrowheads indicate the oocyst capsule. Bar = 1 μm. (**B**) Phase-contrast micrograph of a sporozoite from in vitro culture. (**C**) Fluorescent micrograph of the same sporozoite labeled with an α-circumsporozoite protein monoclonal antibody (*18*). Bar = 10 μm. (**D**) Differential interference contrast micrograph of sporulated oocysts (day 14) produced in vitro. Arrowheads indicate sporozoites. Bar = 30 μm.

and 20 by 60 μm to 25 by 80 μm (elongate) on days 11 to 16. In differentiating oocysts, we observed small refractile droplets similar to those seen in vivo and internal compartmentalization. Sporozoites, either free (Fig. 2B) or still connected to the residual body with a flowerlike appearance (Fig. 2D), were also observed in 12 different flasks from five different experiments, beginning on days 10 to 12. The number of sporozoites peaked on days 14 to 16, but some oocysts continued to produce sporozoites until day 22. These sporozoites expressed circumsporozoite protein antigenicity [Fig. 2C (*18*)].

In experiments in which ookinetes were seeded onto Matrigel but *D. melanogaster* cells were omitted from the culture system, none of the attached oocysts grew beyond 7 to 8 μm and clear signs of deterioration were seen within 4 to 7 days. However, when both L2 cells and Matrigel were excluded from the culture system, virtually all the parasites became round and many adhered to each other, forming large aggregates consisting of hundreds of parasites. About 20% of the clumped parasites grew to a size of 7 to 10 μm and ceased to develop. Nevertheless, in three different experiments rare oocysts continued to grow and produced sporozoites beginning on day 10.

In conclusion, sporozoites of *P. gallinaceum* were observed in cultures maintained under two different sets of in vitro conditions. Of the two, the combination of a Matrigel substrate and L2 cells in suspension probably approximated more closely the natural conditions for development and thus promoted the completion of sporogonic development in a larger proportion of the parasites. In the absence of Matrigel, mutual adhesion of ookinetes may have provided the necessary cue for development, albeit in a very small proportion of the parasites.

One approach for the control of malaria is the introduction into mosquitoes of genes that render them refractory to plasmodial infections. This strategy requires the identification of extrinsic factors involved in the critical developmental stages of the malaria parasite in the mosquito vector (*19*). This paper describes the development of *Plasmodium* in vitro from the blood form to the sporozoite under special conditions that may mimic the signals for development in the mosquito. The factors required for this development can now be more readily identified. Furthermore, the ability to study the development of sporozoites in vitro may help identify necessary components for a still elusive sporozoite vaccine.

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14. L2 cells were grown in Schneider's *Drosophila* medium (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and gentamicin (50 μg/ml), pH 6.8.
15. Oocyst-culture medium: RPMI 1640 (Gibco, catalog no. 320-1875) supplemented with 15% (v/v) heat-inactivated FBS, trehalose (2 g/liter), hypoxanthine (2 mg/liter), 10 mM Hepes, 1 to 2% (v/v) lipoprotein-cholesterol solution (ICN Biomedicals, Costa Mesa, CA; final concentration of cholesterol, 0.2 to 0.8 mg/ml), and gentamicin (50 μg/ml). The pH was adjusted to 7.0; 0.7 ml of medium was used per well (in 24-well cluster) or 3.0 ml per flask (25 cm^2).
16. Matrigel used in these experiments was produced by H. Kleinman (National Institute of Dental Research/NIH). An equivalent product is commercially available through Collaborative Research (Bedford, MA). Matrigel at 4°C was pipetted onto the bottom of the culture vessel [0.25 to 0.3 ml per well (24-well cluster), 0.8 to 1.0 ml per 25-cm^2 cell-culture flask—spread out over approximately 10 to 12.5 cm^2] and allowed to gel for 30 min at room temperature.
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Polymerase II Promoter Activation: Closed Complex Formation and ATP-Driven Start Site Opening

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Studies on bacterial RNA polymerases have divided the initiation pathway into three steps, namely (i) promoter binding to form the closed complex; (ii) DNA melting to form an open complex, and (iii) messenger RNA initiation. Potassium permanganate was used to detect DNA melting by mammalian RNA polymerase II in vitro. Closed complexes formed in a rate-limiting step that was stimulated by the activator GAL4-VP16. Adenosine triphosphate was then hydrolyzed to rapidly melt the DNA within the closed complex to form an open complex. Addition of nucleoside triphosphates resulted in the melted bubble moving away from the start site, completing initiation.

FOR INITIATION OF TRANSCRIPTION to occur, the duplex DNA that composes the start site must open (melt) to expose the nucleotides on the template strand. Melting of DNA requires energy and is a key event in regulation of transcription by bacterial polymerases (*1*), where the open complex containing melted DNA has become a centerpiece for regulatory studies. However, the open complex formed by RNA polymerase II (pol II), which synthesizes mRNA in eukaryotes, has not been unequivocally demonstrated.

Biochemical studies have detected intermediates in the pol II initiation pathway (*2, 3*), including a rapid start complex (*4*) and a phenanthroline-copper–sensitive complex (*5*). Melted DNA within the start site has not been detected in either complex. Both form in the absence of adenosine triphosphate (ATP) hydrolysis, which is required for initiation (*6*). The single-strand selective reagent potas-

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