stained preparations, the same characteristics as *P. inui* found in infected monkeys. The ring stages had large chromatin dots, the trophozoite stages were typically amoeboid, and mature schizonts had 10 to 16 merozoites. Both male and female gametocytes were observed (Fig. 1).

Cultured *P. inui* retained its infectivity to monkeys. Parasites grown in vitro for 35 days were inoculated intravenously into a splenectomized *Saimiri sciureus* monkey (*13*). Eight days later, parasites appeared in smears of peripheral blood, and the animal developed the chronic, benign infection typical of this quartan parasite.

The infective potential of the gametocytes produced in vitro was assessed by feeding cultured parasites through a membrane to *Anopheles freeborni* mosquitoes. Five attempts resulted in one success. Mosquitoes fed on the 34th day of culture were found to have developed oocysts in their guts when dissected 11 days later.

This successful cultivation of a quartan malaria parasite offers promise for future successes with the human parasite *P. malariae*. The possibility of long-term culture of *P. inui* (OS strain), a parasite that can be studied experimentally in various species of monkeys and that is also transmissible to man, will enhance its value as a model of quartan malaria for immunological, biochemical, and ultrastructural investigations.

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12. Trade names and commercial sources are for identification purposes only and do not constitute endorsement by the Public Health Service or by the Department of Health and Human Services.
13. C. C. Campbell (unpublished manuscript) recently demonstrated that *P. inui* (OS strain) could infect *Saimiri sciureus* monkeys.
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9 June 1980

Electrophoretic Injection of a Fluorescent Dye into Giant Mitochondria and Mitoplasts

Abstract. *Studies of the electrical properties of giant mitochondria and mitoplasts with microelectrodes have indicated that there are no significant metabolically dependent membrane potentials. The internal location of the microelectrode has been confirmed by electrophoretically microinjecting the water-soluble dye Lucifer yellow CH into giant mitochondria or mitoplasts.*

Electrophoretic delivery of dyes has been used in electrophysiology to demonstrate the location of the microelectrode tip and to trace cellular processes or connections between cells. One of the first dyes used was Procion yellow M-4RS (*1*). Another group of fluorescent compounds of varied molecular weights has been used to study cell junctions after electrophoretic delivery (*2*). Recently the dye Lucifer yellow CH (dilithium 6-amino-2-[(hydrazinocarbonyl) amino]-2,3-dihydro-1,3-dioxo-1*H*-benz-[*de*]isoquinoline-5,8-disulfonate) was introduced for these purposes (*3*). This dye is highly fluorescent and crosses the neuronal membrane very slowly. Furthermore, Lucifer yellow CH has been shown to diffuse rapidly inside the cells and, at least in neurons, it does not seem to attach significantly to cellular structures.

In the present study Lucifer yellow CH was microinjected electrophoretically into giant mitochondria (*4*, *5*) and giant mitoplasts (*5*) isolated from the livers of mice treated with Cuprizone. The location of the tip of the impaling microelectrode is an important question since previous studies (*5*–*8*) have indicated that the potentials across the membranes of functioning giant mitochondria generally range from about 10 to 20 mV (positive inside) and are metabolically independent. These measurements are not in agreement with one of the major theories of oxidative-phosphorylation, the chemiosmotic theory (*9*), and the location of the electrode tip has been questioned (*10*).

Table 1. Summary of results of microinjection of mitochondria and mitoplasts. Numbers correspond to mitochondria injected.

Preparation	Fluorescence	
	+	–
Mitochondria		
Experimental	55	15
Control (injection at surface)	0	21
Removal of dye by passage of current	1	6
Mitoplasts		
Experimental	15	6
Control	0	7

In the previous studies (*5*–*8*) with microelectrodes of the electrical properties of giant mitochondria isolated from the livers of mice maintained on a diet containing Cuprizone (*4*), the impaled mitochondria have been found to be viable by assays which showed that their ability to accumulate calcium phosphate or phosphorylate adenosine diphosphate was unaltered (*6*, *7*). A number of experimental observations suggest that the impaling microelectrode tips are in the internal space of mitochondria: (i) The membrane potential, usually positive inside, reverses in sign in the presence of valinomycin and becomes dependent on the K^+ concentration of the medium (*5*). (ii) When the mitochondria are impaled with two microelectrodes, the electrical potentials induced can be measured with one microelectrode while current is passed through the other (that is, the two electrodes exhibit cross talk when inside the mitochondrion) (*8*). (iii) The measured resistances and potentials are approximately the same when the mitochondria are stripped of outer membrane and the cristae everted (that is, in mitoplasts) (*6*). (iv) A single potential is recorded when the microelectrode moves through the mitochondrion until it emerges on the other side (*6*).

In our experiments, we filled conventional microelectrodes with 27 m*M* or 65

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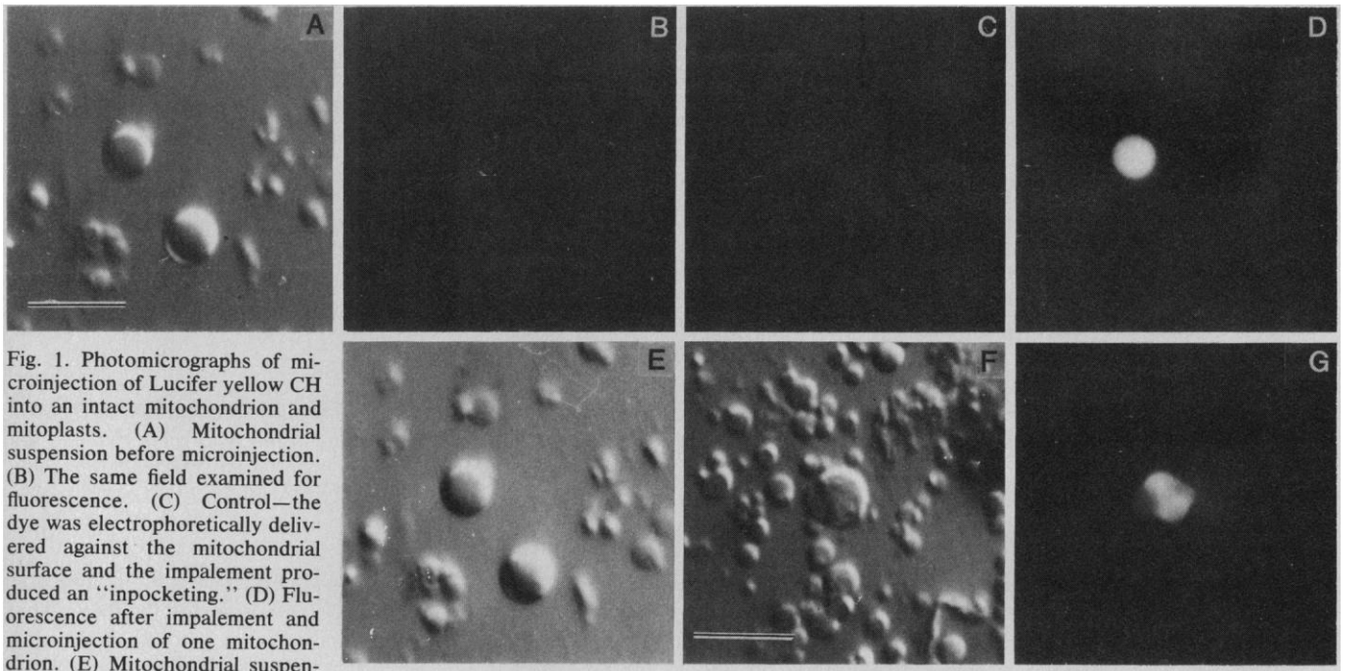

Fig. 1. Photomicrographs of microinjection of Lucifer yellow CH into an intact mitochondrion and mitoplasts. (A) Mitochondrial suspension before microinjection. (B) The same field examined for fluorescence. (C) Control—the dye was electrophoretically delivered against the mitochondrial surface and the impalement produced an "inpocketing." (D) Fluorescence after impalement and microinjection of one mitochondrion. (E) Mitochondrial suspension after microinjection. (F) Mitoplasts. (G) Fluorescence after injection of one mitoplast. Scale bars, 10 μm. Conventional Nomarski optics were used throughout except for the fluorescence portion of the study in which a Zeiss BG12 exciter filter and HBO200 W/4 lamp with a 50 barrier filter were used. Fluorescence was recorded on Eastman Kodak SO-115 film (exposure, 1 to 1.5 minutes), which was developed in D-19 for 8 minutes at room temperature.

m*M* of the lithium salt of Lucifer yellow CH. After impalement, the dye was delivered by 100-msec negative current pulses of 1.4×10^{-9} A. Generally, the pulses were delivered at 200-msec intervals over a period of 1.5 minutes. Mitoplasts were prepared as described in (*5*). The mitochondria or mitoplasts were observed or photographed with a Zeiss Universal microscope.

A chronological sequence of photomicrographs is shown in Fig. 1, and the results of all the experiments are summarized in Table 1. The percentage of mitochondria or mitoplasts exhibiting fluorescence after microinjection (79 and 71 percent, respectively) corresponds approximately to the proportion of successful impalements in the experiments in which we have studied membrane potentials and resistances.

In some experiments the mitochondria were microinjected with the dye, then reimpaled with microelectrodes filled with 2*M* KCl. Negative current (10^{-8} A) was passed through the microelectrode for 10 seconds. This passage of current removes the dye (Table 1). These experiments were carried out to demonstrate that the dye had been taken up reversibly and had not reacted with the mitochondrial components.

The fluorescence of the mitoplasts shows a nonhomogeneous distribution. This is not entirely surprising since the shape of the mitoplasts is not regular, as has also been shown in electron micrographs (*5*). In fact, the locations of the granulations seen with Nomarski optics that might be interpreted as evaginations generally correspond in location to areas of more intense fluorescence. However, a comparison between Fig. 1, F and G, shows that the entire volume of the mitoplast does not appear accessible to the dye. This observation suggests a compartmentalization which is not readily evident from electron micrographs (*5*). It is also possible that, in contrast to the mitochondria we have observed, the mitoplasts are deformed by the impalements. We have not examined this question. In the case of the mitochondria, the fluorescence of the object is invariably homogeneous, an indication that the dye is in the internal space. Photographs of the fluorescence of microelectrode tips filled with the dye indicate that we can resolve fluorescent points as close as 0.5 μm apart. Furthermore, we have seen that in mitoplasts, where the dye does not distribute homogeneously, it is possible to observe a considerable amount of substructure. In the experiments in which the fluorescence was followed over time, it was found to persist for long periods (in some cases for as long as 2 hours).

These experiments show that in either mitochondria or mitoplasts the microelectrodes have entered a space bounded by a relatively impermeable membrane. The homogeneity of the distribution in mitochondria shows that the dye is present in the internal space.

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