dict theoretically. However, by experimentally determining the temperature dependence of either η or τ for a given material, they can be used for measuring temperature.

A practical probe consists of a photoluminescent material attached at the end of a fiber optic bundle. The bundle transmits both the input excitation signal to the photoluminescent material and the luminescent response to a photomultiplier tube light detector.

Figure 2 shows the time-integrated photoluminescent response from two prototype probes, A and B, at temperatures between 20° and 50°C. These probes have an external diameter of 1 mm and use different zinc-cadmium sulfide phosphors as the temperature-sensitive element. The PLT probes were excited with light pulses from a xenon flash. The response was detected with a photomultiplier. The exciting input light signal was separated from the luminescent output signal with appropriate spectral filters. The probe temperature was controlled to 0.1°C through the use of a calibrated water bath. Variations in the response for the system remained within ±1 percent for measurements taken over several weeks. Drift in the probe calibration, if any, remained below this noise level. With the present system, measurement noise of ±1 percent corresponds to a temperature resolution of approximately 0.3°C for probe A and 0.4°C for probe B. Variations in the input light pulse intensity are a major source of fluctuations in the probe response. Methods to eliminate or compensate for these variations are being developed, and we expect that resolution can be improved to 0.1°C. The temperature sensitivity and resolution can also be improved by an optimum choice of photoluminescent material, as is demonstrated by the increased resolution of probe A with respect to probe B (Fig. 2).

Temperature probes designed for use in hyperthermia treatments on humans should be less than 1 mm in size, structurally sturdy, thermally stable, and minimally perturbed in EM fields. These photoluminescent temperature probes demonstrate the potential for achieving the objectives.

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4 October 1979

Vertebrate Cells Express Protozoan Antigen After Hybridization

Abstract. *Epimastigotes, the invertebrate host stage of* Trypanosoma cruzi, *the protozoan parasite causing Chagas' disease in man, were fused with vertebrate cells by using polyethylene glycol. Hybrid cells were selected on the basis of* T. cruzi *DNA complementation of biochemical deficiencies in the vertebrate cells. Some clones of the hybrid cells expressed* T. cruzi*-specific antigen. It might be possible to use selected antigens obtained from the hybrids as vaccines for immunodiagnosis or for elucidation of the pathogenesis of Chagas' disease.*

Trypanosoma cruzi, an obligate intracellular protozoan parasite, causes Chagas' disease, an incurable human disease prevalent in South and Central America. Attempts to produce an effective vaccine against infection have not been successful; dead organisms provide only partial protection against subsequent challenge (*1*). The use of avirulent organisms as living vaccines involves the risk of reversion to virulence and subsequent low-level, chronic infection. Selected antigens of the infectious agent would seem more appropriate for the preparation of vaccines. Unfortunately, the isolation and purification of selected antigens from *T. cruzi* as well as other parasites presents serious logistic problems. If somatic cell hybrid technology could be used for the production of parasite antigen, as it has been for the production of monoclonal antibodies (*2*), a major problem in antigen production might be overcome. In this report, we describe fusion between the epimastigote stage of *T. cruzi* and two different mammalian cell types and the production of hybrids that express parasite antigen.

Epimastigotes of the Tulahuen strain of *T. cruzi* were grown at 23°C in liver-infusion tryptose (LIT) medium (*3*) supplemented with 10 percent fetal calf serum and, per milliliter, 20 μg of hemin, 100 U of penicillin, and 100 μg of streptomycin. The vertebrate cells, BESM (*4, 5*), P3-x63Ag8 (*2*), and hybrids were maintained according to routine tissue culture procedures.

Two fusion methods were used to allow studies of both the morphological aspects of the fusion of *T. cruzi* and vertebrate cells and the production of *T. cruzi* antigen-expressing vertebrate cell clones.

A modification (*5*) of the pancake technique of O'Malley and Davidson (*6*) was used to study the morphology of *T. cruzi* and vertebrate cell fusion. This consisted of centrifuging epimastigotes onto monolayers of BESM cells, treating the cells with polyethylene glycol (PEG), and then observing the resultant products by light microscopy (*7*).

In Fig. 1, A and B, the flagellum of a parasite is shown extending from the surface of a BESM cell at approximately 20 minutes after treatment with the PEG solution. Flagella (one to five per heterokaryon) exhibited typical, sinusoidal motion for up to 3 to 4 hours, after which time they became less active and more difficult to observe. The anisotropic nature of the parasite's kinetoplast, an extranuclear DNA-containing organelle, enables it to be detected by polarized light (*8*). During the early stages of fusion, the kinetoplast was observed in the vertebrate cell cytoplasm either close to or some distance from the proximal end of the flagellum (Fig. 1, C and D). Neither the limiting membrane nor the nucleus of the parasite could be visualized.

To identify the parasite nucleus in the heterokaryon, we repeated the above experiments using [^{3}H]thymidine-labeled epimastigotes (*9*). An example of such an epimastigote is shown in Fig. 1E. Figure 1F shows a portion of a binucleate *T. cruzi* × BESM heterokaryon with a labeled parasite nucleus and an unlabeled BESM nucleus. Only the nucleus of the parasite could be observed. The remaining structures of the parasite could not be found.

Heterokaryons remained viable, as in-

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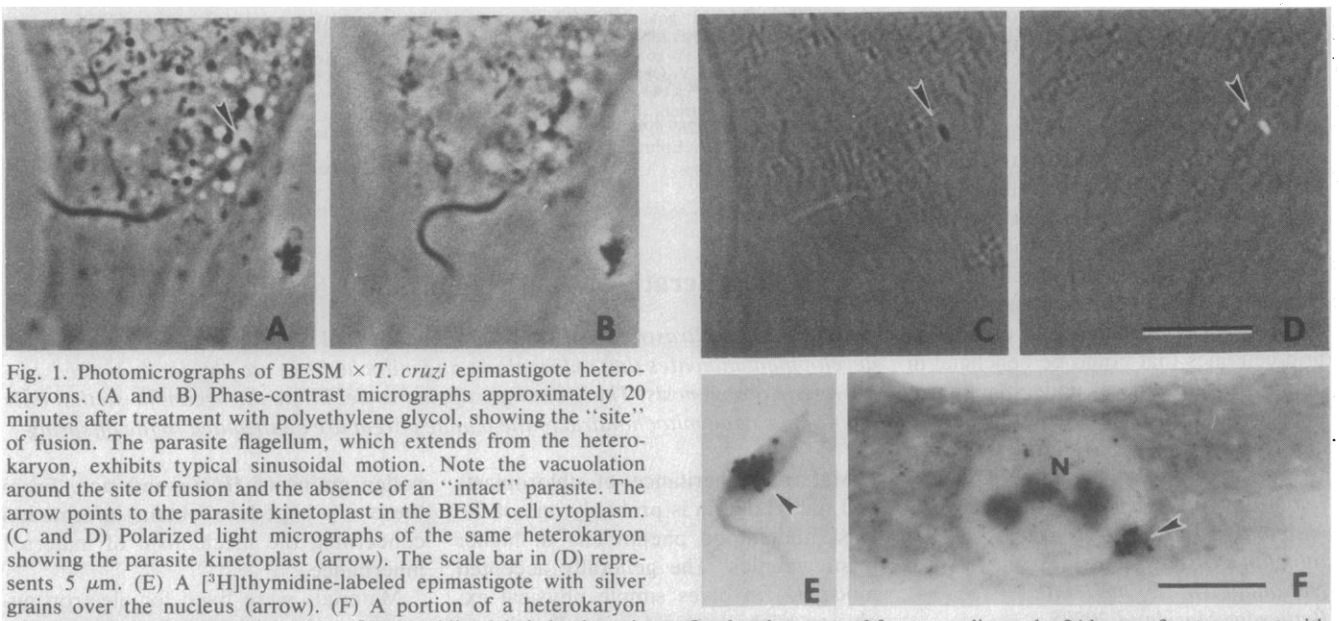

Fig. 1. Photomicrographs of BESM × *T. cruzi* epimastigote heterokaryons. (A and B) Phase-contrast micrographs approximately 20 minutes after treatment with polyethylene glycol, showing the "site" of fusion. The parasite flagellum, which extends from the heterokaryon, exhibits typical sinusoidal motion. Note the vacuolation around the site of fusion and the absence of an "intact" parasite. The arrow points to the parasite kinetoplast in the BESM cell cytoplasm. (C and D) Polarized light micrographs of the same heterokaryon showing the parasite kinetoplast (arrow). The scale bar in (D) represents 5 μm. (E) A [^{3}H]thymidine-labeled epimastigote with silver grains over the nucleus (arrow). (F) A portion of a heterokaryon formed by fusing a BESM cell with a [^{3}H]thymidine-labeled epimastigote, fixed and processed for autoradiography 24 hours after treatment with polyethylene glycol. The arrow indicates the labeled epimastigote nucleus adjacent to the unlabeled BESM nucleus (*N*). Scale bar, 10 μm.

dicated by cellular migration, for at least 3 days, after which time cultures were overgrown because of the faster growth rate of unfused cells. Cells infected with *T. cruzi* were not observed in any of these cultures.

A second procedure for fusion, a modification (*10*) of the suspension technique (*11*), was used to obtain hybrid cell lines. Epimastigotes were fused to P3-x63Ag8 cells and selection for hybrids was carried out in Dulbecco's modified Eagle's minimal essential medium containing hypoxanthine, aminopterine, and thymidine (HAT) (*12*) at 37°C. Because of an enzyme deficiency, P3-x63Ag8 cells are HAT-sensitive (*2*). Epimastigotes, however, are HAT-resistant and grow normally at their permissive temperature of 23°C. At 37°C, epimastigote growth is inhibited (*13*, *14*). Consequently, the procedure of growing fusion products at 37°C in HAT medium allows for the selection of *T. cruzi* × P3-x63Ag8 hybrids.

At about 3 weeks after fusion, stocks of hybrid cells were pooled and cloned by the limiting dilution technique. The cloning procedure was repeated twice more and clones were grown at 37°C. The resultant hybrid cell cultures were tested for the expression of *T. cruzi*–specific antigen by an indirect immunofluorescent antibody assay (*15*). Three hybrid cultures expressed *T. cruzi* antigen even after 14 weeks of serial cultivation after fusion. A demonstration of expression of *T. cruzi* antigen by one of these hybrids is shown in Fig. 2. None of the hybrid clones were infected with *T. cruzi*.

The mechanism by which the genetic material of *T. cruzi* is incorporated into the vertebrate cell nucleus is unknown. The fusion of vertebrate cells results in binucleate heterokaryons in which admixing of genetic material occurs during the first mitotic division after fusion (*16*). Hybridization can then be demonstrated by karyotypic analysis (*17–19*). However, the nuclear DNA of *T. cruzi* is not organized into chromosomes typical of eukaryotic cells, and the nuclear membrane remains intact during mitosis (*20*). As karyotypic analysis is not possible with this organism, it is proposed that the expression of *T. cruzi* antigen by the hybrid cells after 14 weeks of continuous culture demonstrates the presence of functional parasite DNA.

The antigen or antigens expressed by the *T. cruzi* × P3-x63Ag8 hybrid clones have not been characterized. However, a variety of selected antigens produced by the hybrid clones may be used for studies on vaccination, immunodiagnosis, or immunopathogenesis of Chagas' disease. Serums or antibodies from patients with Chagas' disease enhance the penetration of vertebrate cells by *T. cruzi* in vitro (*21*). Identification of the antigens responsible for the induction of the antibodies that mediate this enhancement is important especially for studies concerning vaccine production. Ultimately, it may be possible to use antigens from *T. cruzi* × vertebrate cell hybrids as noninfectious vaccines. In this respect, selection of antigens with pro-

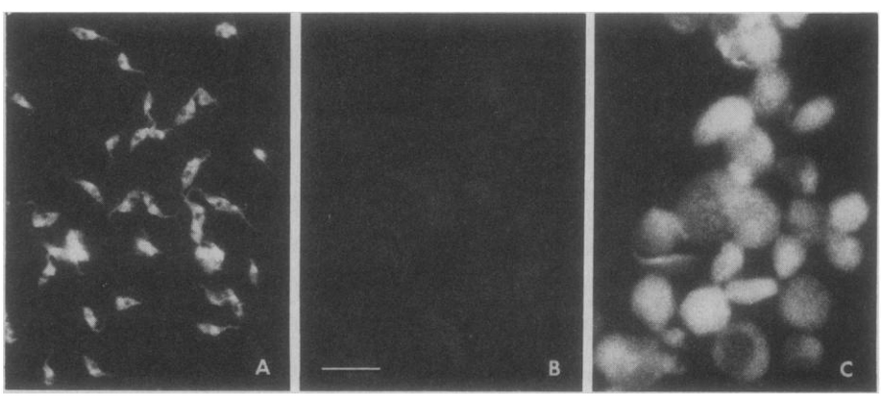

Fig. 2. Immunofluorescent staining of *T. cruzi* antigen. The cells were air-dried, fixed (1.5 percent formaldehyde in PBS for 15 minutes at 0°C), incubated with rabbit antiserum to *T. cruzi*, and stained with FITC-conjugated goat antiserum to rabbit globulin. (A) Epimastigotes of *T. cruzi* showing a positive reaction to antiserum to *T. cruzi*. (B) P3-x63Ag8 cells showing a negative reaction to antiserum to *T. cruzi* serum. (C) A hybrid of *T. cruzi* × P3-x63Ag8 showing a positive reaction to antiserum to *T. cruzi*. Scale bar, 10 μm.

tective activity and elimination of antigens with harmful effects should be possible.

To our knowledge, this is the first description of successful fusion and hybrid formation between invertebrate and vertebrate cells. However, the ability of *T. cruzi* to fuse with mammalian cells may not be unique; it may be possible to fuse other invertebrate cells, such as protozoans or cells from metazoan parasites, to vertebrate cells and obtain hybrid cell lines that could be beneficial in improving our general understanding of parasite-induced infectious diseases.

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10. A pellet of 6×10^6 P3-x63Ag8 cells and 7×10^7 epimastigotes was resuspended in 0.5 ml of a 50 percent (weight to volume in PBS) solution of PEG 1540, mixed for 1 minute, and then diluted with Dulbecco's modified Eagle's minimal essential medium supplemented with 10 percent fetal calf serum. After overnight incubation, the medium was supplemented with $10^{-4}M$ hypoxanthine, $4 \times 10^{-7}M$ aminopterine, $1.6 \times 10^{-5}M$ thymidine, and $3 \times 10^{-6}M$ glycine (*12*).
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Organelle Alteration as a Mechanism for Maternal Inheritance

Abstract. *An ultrastructural study of pollen-derived plants and normal microspore development indicates that chloroplasts and mitochondria are physically altered during microsporogenesis. These changes appear to debilitate the organelle so that only chloroplasts and mitochondria of the female parent are contributed to the offspring.*

Maternal inheritance of chloroplasts and mitochondria is probably one of the least understood phenomena in higher plant genetics. The generally accepted model (*1*) involves simple physical exclusion of the paternal organelles due to the small size of the male generative cell. Physical exclusion rather than alteration of the chloroplasts and mitochondria was also indicated by the fact that apparently normal green haploid plants, supposedly with normal plastids, were obtained from tissue culture of uninucleate pollen in a number of different species (*2*).

However, in *Oryza*, *Hordeum*, and *Triticum* (*3*), white haploid plantlets from uninucleate pollen are frequently obtained, indicating that the chloroplast may be altered prior to the formation of vegetative and generative cells. To investigate this apparent alteration, we did an ultrastructural study of a collection of albino rice plantlets and of developing pollen grains of *Hosta* and rice. From these we hoped to obtain information concerning the mechanism of maternal inheritance.

Materials were fixed for electron microscopy by the standard glutaraldehyde-osmium technique, although for tissue culture plantlets 0.3*M* ribonuclease-free sucrose and 0.5 percent (weight to volume) caffeine were added to buffered glutaraldehyde. Specimens were subsequently handled as described by Travis *et al.* (*4*). Plant materials used were a rice albino from U.S. Department of Agriculture (USDA) stocks, eight rice albinos from S.-C. Woo in Taiwan (all albino plantlets were obtained from uninucleate pollen), and developing pollen grains of rice and *Hosta*.

The leaves of white rice plantlets derived from uninucleate pollen grains show structures that are recognizable as plastids by several criteria. Observed

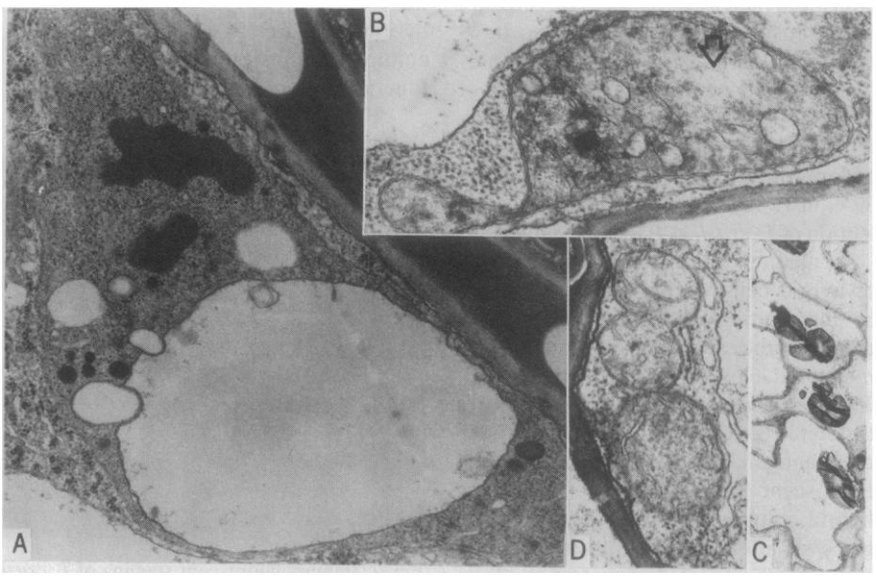

Fig. 1. Ultrastructural aspects of some albino plantlets of rice. (A) Plastid from albino obtained from the USDA (×25,000). (B) Plastid from one of the albino strains obtained from S.-C. Woo, showing a distinct fibrillar area (arrow) (×18,000). (C) Group of cells from an albino rice strain where the mesophyll cells accumulate starch in the plastids (×4,500). (D) Mitochondrial aggregate from an albino rice strain obtained from Woo (×25,000).

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