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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References and Notes

1. F. C. Goble, in *Immunity to Parasitic Diseases*, G. J. Jackson, R. Herman, I. Singer, Eds. (Appleton-Century-Crofts, New York, 1970), vol. 2, p. 597.
2. G. Kohler and C. Milstein, *Nature (London)* **256**, 495 (1975).
3. G. J. Boné and M. Steinert, *ibid.* **178**, 308 (1956).
4. J. A. Dvorak and T. P. Hyde, *Exp. Parasitol.* **34**, 268 (1973).
5. The BESM (bovine embryo skeletal muscle) cells (2.5×10^4 per milliliter) were grown on round cover glasses (25 mm in diameter), and then 3×10^7 to 4×10^7 epimastigotes in phosphate-buffered saline (PBS) were added on top of the BESM cover glass culture supported by a Delrin plug in a centrifuge tube and centrifuged at 600*g* for 10 minutes. The supernatant fluid was removed and the cover glass placed for 1 minute into a PEG solution [50 percent PEG (molecular weight 1540) in PBS], rinsed, and placed in a Dvorak-Stotler culture chamber (*7*) for observation.
6. K. A. O'Malley and R. L. Davidson, *Somatic Cell Genet.* **3**, 441 (1977).
7. J. A. Dvorak and W. F. Stotler, *Exp. Cell Res.* **68**, 144 (1971).
8. J. A. Dvorak, unpublished data.
9. An epimastigote culture, in the exponential phase of growth, was labeled with 10 μCi of [methyl-^{3}H]thymidine per milliliter (Amersham; specific activity, 5.0 Ci/mmole) for 1 hour, washed twice by centrifugation, reincubated at 23°C for 9 hours, and exposed to [methyl-^{3}H]thymidine again for 1 hour. This procedure labeled approximately 100 percent of the parasite population.
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*).
11. R. L. Davidson and P. S. Gerald, *Somatic Cell Genet.* **2**, 165 (1976).
12. J. W. Littlefield, *Science* **145**, 709 (1964).
13. At 23°C there is a 20-fold increase in the number of epimastigotes grown over a period of 8 days in either normal or HAT medium. At 37°C, there is only a twofold increase in the number of epimastigotes, grown over a period of 6 days.
14. J. F. Fernandes and O. Castellani, *Exp. Parasitol.* **18**, 195 (1966).
15. Rabbits were inoculated subcutaneously (multiple sites) with 1 ml of a mixture containing 0.5 mg of protein (disrupted epimastigotes) and 0.5 ml of complete Freund's adjuvant on days 1 and 14. Rabbit antiserum to *T. cruzi* and normal rabbit serum were absorbed with lyophilized P3-x63Ag8 cells. Fluorescein isothiocyanate-(FITC)-conjugated goat antiserum to rabbit globulin (Gibco) was absorbed with lyophilized epimastigotes and P3-x63Ag8 cells.
16. T. Yamanaka and Y. Okada, *Exp. Cell Res.* **49**, 461 (1968).
17. G. Barski, S. Sorieul, F. Cornefert, *J. Natl. Cancer Inst.* **26**, 1269 (1961).
18. S. Sorieul and B. Ephrussi, *Nature (London)* **190**, 653 (1961).
19. M. C. Weiss and H. Green, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1104 (1967).
20. W. de Souza and H. Meyer, *J. Protozool.* **21**, 48 (1974).
21. J. A. Dvorak and G. A. Schmunis, Fourth International Congress on Parasitology, Warsaw (1978), sect. E, abstr. 26.

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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

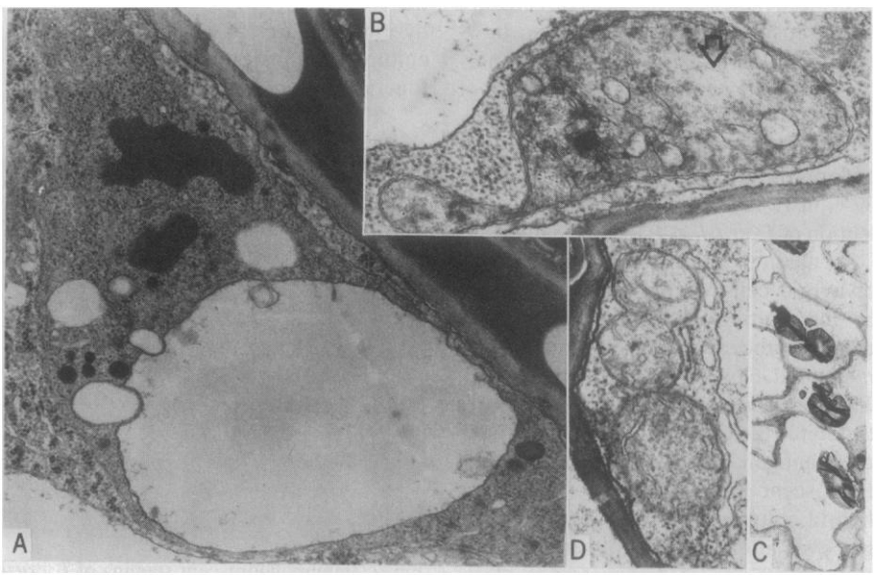

Fig. 1. Ultrastructural aspects of some albino plantlets of rice. (A) Plastid from albino obtained from the USDA (×25,000). (B) Plastid from one of the albino strains obtained from S.-C. Woo, showing a distinct fibrillar area (arrow) (×18,000). (C) Group of cells from an albino rice strain where the 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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structures typical of all mesophyll plastids, which are found in the white rice plastids, are double membranes, osmiophilic globules, and ribosomes (Fig. 1, A and B). Compared to the green wild-type mesophyll plastid, these white plastids have a much less complex thylakoid system, consisting of thylakoid circles of varying dimensions. Guard cell plastids of the USDA strain as well as the mesophyll plastids of one of the strains obtained from Woo contain starch grains (Fig. 1C). Since plastids are defined as organelles with the ability to accumulate starch (*5*), these organelles appear to be, at least in a biochemical sense, true plastids.

Because structures recognizable as ribosomes are present in these plastids, one must assume that at least partially functional chloroplast DNA is present. Fibrillar areas of DNA (Fig. 1B) are also evidence for the presence of DNA. The white color of the plantlets may be due to photooxidation of the pigments as they are produced, because the abnormal thylakoid system may not allow normal pigment-pigment and pigment-protein associations (*6*).

Although Sun (*7*) states that the cause of albinism in the plantlets is lack of ribosomes, the presence of such structures in some of the strains investigated indicates that this is not the reason for the white color of these plantlets. Likewise, the variable ultrastructure of these plastids (Fig. 1, A, B, and C) indicates that a mechanism more akin to mutation or restriction is responsible for the plastid alterations. Aneuploidy was eliminated as a cause of albinism, at least in *Triticum*, by a karyological study (*3*).

It is more difficult to assess ultrastructural irregularities in mitochondria than in plastids because of the relatively simple ultrastructure of the mitochondria. However, mitochondrial aggregates were consistently observed in these strains (Fig. 1D). Those seen in pollen plantlets appear to share a common outer membrane but have a distinct inner membrane. Similar mitochondrial aggregates are noted in animal sperm (*8*).

Several workers (*9*) found that mutant plastids in species that normally inherit biparentally, which were strongly debilitating to the plant, were not transmitted with success. It is likely that the observed alterations of chloroplasts and mitochondria would prevent them from being transmitted. The strict maternal inheritance of plastid traits observed in rice (*10*) is consistent with this hypothesis.

Perhaps the most important observation in this work is that of modification of both the mitochondrion and the chloroplast before the formation of the generative cell, since only uninucleate pollen was used as a plantlet source (unless these are artifacts of tissue culture, as discussed below). Thus, even if physical exclusion is a factor, the organelles that are excluded are debilitated ones.

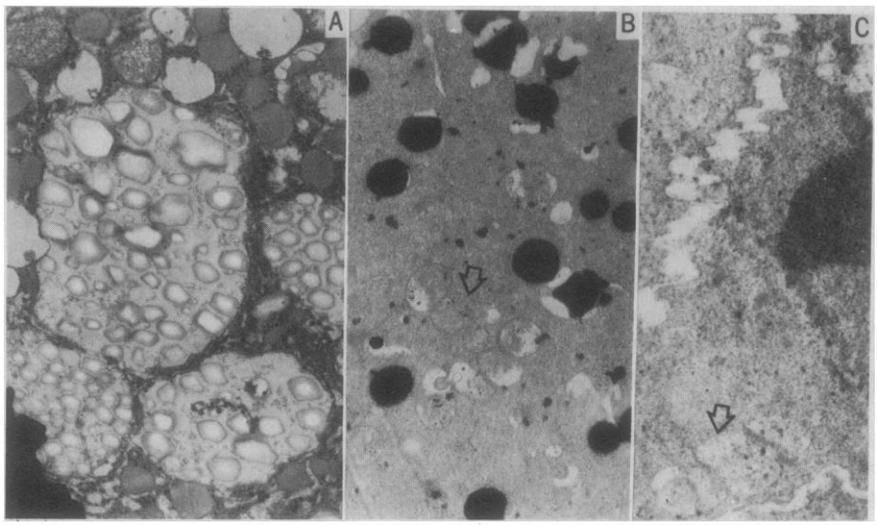

Fig. 2. Ultrastructural changes in developing *Hosta* pollen. (A) Large starch-filled plastids in pollen before the mitosis, following meiosis (×16,000). (B) Portion of the vegetative cell of a pollen grain just before anthesis with a mitochondrial aggregate of seven (arrow) and no recognizable plastids (×10,000). (C) Section of generative cell of *Hosta* with mitochondrial aggregate (arrow) (×20,000).

When the pollen development of *Hosta* and rice was examined, similar kinds of paternal organelle alterations were observed; this indicates that the pollen-derived plantlets were not artifacts but gave an accurate picture of the state of the organelles at the time when the pollen was collected. The alterations observed in *Hosta* were the most extreme ones among the species we examined and will be dealt with in this report. Because of the large size of the anthers, it was also easier to collect *Hosta* in the early developmental stages.

Pollen mother cells and uninucleate pollen of *Hosta* contain huge starch-filled plastids occupying a large percentage of the pollen area (Fig. 2A). However, just before and after meiosis, plastids appear only as remnants or are absent altogether (Fig. 2B). The mitochondria clump into aggregates of as many as seven (Fig. 2B), with membrane alterations characteristic of autophagy (*11*). Mitochondrial aggregates are noted in the generative cell (Fig. 2C) as well as the vegetative. These data indicate that chloroplasts and mitochondria may be physically altered in the process of microsporogenesis, preventing them from being transmitted. Because mitochondria are found in the generative cell, the idea that physical exclusion explains mitochondrial maternal inheritance, as noted in maize (*12*), appears erroneous. Since it is believed that mitochondria aggregate in response to leakiness of the membranes (*13*), our ultrastructural data indicate that the mitochondria are truly altered. Surely, the small size of the generative cell limits the number of organelles that can be passed on, but these studies show that other alterations are more likely to be involved in the mechanism of maternal inheritance, especially for mitochondria.

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References and Notes

1. R. Hagemann, in *Genetics and Biogenesis of Chloroplasts and Mitochondria*, T. Bucher, W. Neupert, W. Sebald, W. Werner, Eds. (North-Holland, Amsterdam, 1976), p. 331; J. T. O. Kirk and R. A. E. Tilney-Bassett, *The Plastids* (North-Holland, Amsterdam, 1978); N. W. Gillham, *Organelle Heredity* (Raven, New York, 1978).
2. T. Nilsson-Tillgren and P. von Wettstein-Knowles, *Nature (London)* **227**, 1265 (1970); N. Sunderland, in *Haploids in Higher Plants—Advances and Potential*, K. J. Kasha, Ed. (Univ. of Guelph, Guelph, 1974), p. 91.
3. C.-C. Chen, *In Vitro* **13**, 484 (1977); G. W. Schaeffer, P. S. Baenziger, T. Worley, *Crop. Sci.* **19**, 697 (1979).
4. D. M. Travis, K. D. Stewart, K. G. Wilson, *Theor. Appl. Genet.* **46**, 67 (1975).
5. N. P. Badenhuizen, *The Biogenesis of Starch Granules in Higher Plants* (Meredith, New

York, 1969); R. Salema and I. Abreu, *Bol. Soc. Broteriana* **46**, 259 (1972).
6. R. Knoth, *Biol. Zentralbl.* **94**, 513 (1975); M. B. Fields, K. C. Vaughn, K. G. Wilson, *J. Hered.* **69**, 263 (1978).
7. C.-S. Sun, *Sci. Sin.* **17**, 627 (1974).
8. P. Favard and J. Andre, in *Comparative Spermatology*, B. Baccetti, Ed. (Academic Press, New York, 1970), p. 415.
9. F. Schotz, *Biol. Zentralbl.* **93**, 41 (1974); R. N. Stewart, P. Semeniuk, H. Dermen, *Am. J. Bot.* **61**, 54 (1974).
10. B. P. Pal and S. Ramanujam, *Indian J. Agric. Sci.* **14**, 170 (1941).
11. P. Matile, in *Plant Biochemistry*, J. Bonner and J. Varner, Eds. (Academic Press, New York, 1976), p. 189.
12. M. Conde, D. R. Pring, C. S. Levings, *J. Hered.* **70**, 2 (1979).
13. K. T. Tokuyasu, *J. Ultrastruct. Res.* **53**, 93 (1975).
14. We thank M. J. Powell and K. D. Stewart for their helpful discussions and S.-C. Woo for his kind contribution of albino plantlets. This work was supported in part by grants from the Miami University Faculty Research Committee.

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Magnesium Deficiency Produces Spasms of Coronary Arteries: Relationship to Etiology of Sudden Death Ischemic Heart Disease

Abstract. *Isolated coronary arteries from dogs were incubated in Krebs-Ringer bicarbonate solution and exposed to normal, high, and low concentrations of magnesium in the medium. Sudden withdrawal of magnesium from the medium increased whereas high concentrations of magnesium decreased the basal tension of the arteries. The absence of magnesium in the medium significantly potentiated the contractile responses of both small and large coronary arteries to norepinephrine, acetylcholine, serotonin, angiotensin, and potassium. These data support the hypothesis that magnesium deficiency, associated with sudden death ischemic heart disease, produces coronary arterial spasm.*

Several recent investigations point to a causal relation between decreased magnesium ion (Mg^{2+}) content of cardiac muscle and coronary arteries and mortality from (nonocclusive) sudden-death ischemic heart disease (SDIHD), the incidence of which is highest in geographic areas with soft drinking water or magnesium-poor soil (*1-5*). Of the minerals that are deficient in soft water, magnesium is the only element that has been found to be lowered in the cardiac muscle of SDIHD victims (*1-3, 5*). Acute hypomagnesemia in animals and man is often associated with increases in blood pressure and in peripheral vascular resistance in several regional circulatory systems (*6, 7*). Artificial lowering of the Mg^{2+} content of isolated (noncardiac) vessels from rats, rabbits, piglets, and dogs induces rapid, potent contractile responses (*8-10*). Acute hypermagnesemia inhibits the spontaneous tone of arteries and veins (*8-10*). Thus, there is evidence that extracellular Mg^{2+} plays a critical role in the regulation of vasomotor tone.

A positive correlation between mortality rates from SDIHD and the estimated high ratio of calcium to magnesium in myocardial tissue has been demonstrated (*3, 4*). Although many tissues of the body have been shown to be resistant to Mg^{2+} depletion, heart tissue and coronary vessels have a significantly reduced Mg^{2+} content in cases of SDIHD (*1, 3, 5*). It was therefore suggested recently that SDIHD mortality could be due to the direct effects of a hypomagnesemic state on coronary vascular tone (*11*). The hypomagnesemia could produce progressive vasoconstriction, vasospasm, and ischemia, which, given time, would lead to SDIHD. To investigate the possibility that vasospasm can be produced by Mg^{2+} deficiency, we determined the influence of sudden magnesium withdrawal and hypermagnesemia (4.8 m*M*) on vascular tone and vasoactive drug-induced responses in isolated coronary arteries of the dog.

Mongrel dogs of either sex weighing 10 to 20 kg were anesthetized with pentobarbital sodium (30 mg/kg). The hearts were removed immediately and coronary arteries were isolated. Because of possible segmental differences in coronary arterial reactivity (*12*), we examined four different coronary inflow vessels: left coronary [outer diameter (O.D.), 1 to 2 mm], circumflex (O.D. 1 to 2 mm), left coronary branch (O.D. < 1 mm), and right coronary branch (O.D. < 1 mm). Helical strips, cut from segments of these coronary arteries, were 20 to 25 mm long by 0.5 to 1.0 mm wide. These were suspended isometrically under 1 g of tension (circumflex, left coronary arteries) or 0.5 g of tension (left and right coronary branch arteries) and incubated in 20-ml muscle chambers containing Krebs-Ringer bicarbonate solution (composition in millimoles per liter: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 10; and $NaHCO_3$, 25) at 37°C through which a mixture of O_2 (95 percent) and CO_2 (5 percent) was bubbled. Force of contraction was measured with Grass FT-03 force-displacement transducers and recorded on a Grass model 7 polygraph. Two hours after the preparations were incubated, under tension, the effects of extracellular Mg^{2+} concentration ($[Mg^{2+}]_o$) and vasoactive drugs were examined. The arteries were sequentially exposed to normal (1.2 m*M*), low (0 m*M*), and high (4.8 m*M*) concentrations of magnesium.

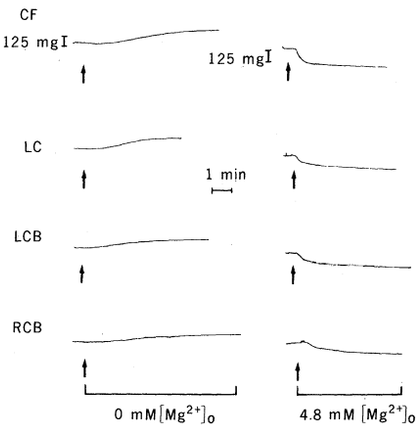

Fig. 1. Influence of extracellular Mg^{2+} on basal tension of canine circumflex (*CF*), left coronary (*LC*), left coronary branch (*LCB*), and right coronary branch (*RCB*) arterial strips. The left panel shows results obtained in a Mg^{2+}-free environment; the right panel indicates results obtained in 4.8 m*M* $[Mg^{2+}]_o$. The vertical bar represents tension (125 mg); time marker, 1 minute. Arrows indicate points at which the normal Krebs-Ringer medium containing 1.2 m*M* $[Mg^{2+}]_o$ was switched to the experimental concentration of $[Mg^{2+}]_o$.

Sudden withdrawal of extracellular Mg^{2+} resulted in rapid, increased tension development in all coronary vessels tested (Fig. 1 and Table 1). In contrast, a sudden increase in extracellular Mg^{2+} (4.8 m*M*) resulted in rapid relaxation of basal tension in all coronary arteries (Fig. 1 and Table 1). Thus, extracellular Mg^{2+} appears to be able directly to alter coronary arterial baseline tension or tone. A similar influence of $[Mg^{2+}]_o$ on vasomotor tone was previously demonstrated in isolated rat and piglet arteries, rabbit aortas, and rat arterioles and portal veins (*8-10*). These effects of $[Mg^{2+}]_o$ cannot be attributed to osmolarity differences, inhibition of Na^+- and K^+-dependent adenosinetriphosphatase activity, or release (or inhibition of release) of endogenous neurohumoral agents from the arterial wall (*7, 8, 10, 13, 14*).

In addition to increasing tone, the withdrawal of Mg^{2+} potentiated the constrictor actions of vasoactive substances such as angiotensin, serotonin, norepinephrine, acetylcholine, and potassium in all coronary arteries (see Fig. 2, Table 2). The order of increase in tension to the vasoactive agents was acetylcholine > norepinephrine > serotonin > K^+ > angiotensin in all types of coronary vessels studied. These enhanced responses,

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