Antimycoplasmal Antibiotics and Hybrid Sterility in Drosophila paulistorum

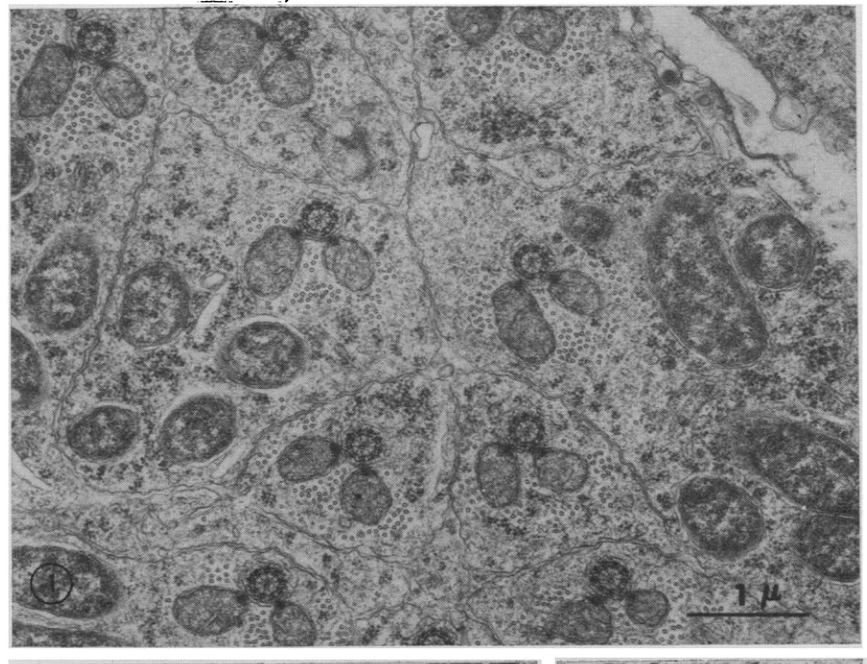
Abstract. *Male hybrids between the Santa Marta and Mesitas strains of* Drosophila paulistorum *are sterile. The sterility can be partially alleviated by treatment of the Santa Marta mothers with the antimycoplasmal antibiotics tylosin tartrate and tetracycline hydrochloride. The proportions of hybrid males in which there are mature motile spermatozoa are higher in the progenies of drug-treated mothers than in the controls. Electron microscopy reveals mycoplasma-like bodies in the untreated controls, but not in the sons of the treated females. The mycoplasma-like symbionts appear to be implicated in the production of the sterility in the hybrid males.*

Crosses between semispecies of *Drosophila paulistorum* produce sterile male hybrids in the F_1 and in backcross generations (*1*). In some crosses, such as between Santa Marta females and Mesitas males, the factor responsible for the sterility can also be transmitted by injection (*2*) and partially suppressed by heat shocks (*3*). Santa Marta larvae were grown on media with the antibiotics toyocamycin nucleoside and gliotoxin (100 μg/ml). Adult females obtained from such larvae were crossed to Mesitas males; among their sons, the proportion of males with motile spermatozoa was higher than in the progenies of untreated mothers (*4*).

Ultrastructural analysis was made of the testes of sterile hybrid males. Spermatid bundles undergo degenerative changes involving disarrangement of the axial filament complex and the associated mitochondrial derivatives (*5*). Bodies resembling mycoplasma are seen within and between the degenerating spermatid bundles, intra- as well as extracellularly, and elsewhere inside the testicular membrane (Figs. 1 and 2). Each mycoplasma-like body is enclosed in a membrane and shows a central reticular network and peripheral ribosome-like granules. Testes of fertile nonhybrid males of *Drosophila paulistorum* have similar mycoplasma-like inclusions, but far less numerous ones.

Attempting to test the possible causal relations between the presence of mycoplasma-like bodies and the sterility of the hybrid males, we have examined the effects of other antibiotics, namely, tylosin tartrate (Lilly: Tylan) and Achromycin (Lederle: tetracycline hydrochloride). Tylosin is effective against avian and mammalian mycoplasma in broth cultures (*6*), against a mycoplasma contaminant in a murine leukemia tissue culture (*7*), and against the arthropod-borne mycoplasma-like causative agent of the plant disease aster yellows (*8*).

A solution of tylosin (100 μg/ml) in water was prepared; 0.3 ml was added to each 250-ml culture bottle of drosophila food, namely, Ohba's medium (8 percent) which consists of distilled water, agar, dry dead yeast, and Tegosept, an antimold agent (*9*). The Santa Marta females and Mesitas male flies were put on this drug-impregnated medium. Their progenies were kept on this food through three larval instars and pupation until the hatching of the hybrid F_1 adults. Thereafter all subsequent generations were raised on the ordinary drug-free drosophila culture medium devised by Spassky (*10*). Tetracycline was administered in precisely the same way that tylosin was; it was



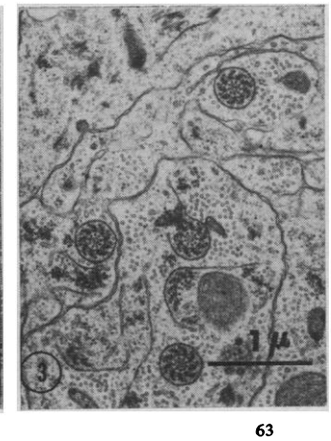


Fig. 1. Cross section of a sperm bundle in the testis of a sterile hybrid. Arrangement of the axial filament complex and flanking mitochondrial derivatives is normal. Clusters of mycoplasma-like bodies are observed between and within the spermatid bundles (× 23,500). Fig. 2. Clusters of mycoplasma-like bodies in the lumen of the testis of a sterile hybrid (× 27,500). Fig. 3. Developing spermatids in the testes of a tylosin-treated F_1 hybrid showing disturbances of the radial symmetry in the axial filament complex. No mycoplasma-like structures are visible (× 20,000).

Table 1. The effects of tylosin tartrate and tetracycline on hybrids between the Santa Marta (SM) and Mesitas (M) strain of *Drosophila paulistorum*. Samples of 100 males were dissected in each generation in each experiment.

Cross	Percentage of males with motile sperm		
	Tylosin	Tetracycline	Controls
F_1 of SM ♀♀ × M ♂♂	0	40	0
BC_1 (SM × M) F_1 ♀♀ × M ♂♂	35	20	8–10

effective against the mycoplasma-like structures associated with plants infected with mulberry dwarf disease (*11*) and against aster yellows disease (*8*). Table 1 reports the percentages of the males in the F_1 and in the first backcross generation showing motile sperm upon dissection. In the controls, the mothers of these males had developed as larvae on food free of antibiotics, and in the experimental series the mothers were given antibiotics in their food. Treatment with tetracycline, but not with tylosin, results in the appearance of motile sperm in a part of the F_1 generation hybrid males. Both tetracycline and tylosin increase the frequency of motile sperm in backcross males. To test the fertility of the males, 30 cultures were made with eight females and eight males from each experiment reported in Table 1. No fertile cultures were obtained in the control experiments or in the F_1 hybrids treated with tylosin. In the other experiments, where many males had motile sperm, some of them were also fertile. However, the backcross males in the control experiments were all sterile, although some of them had motile sperm, as reported (*4, 12*).

Ultrastructurally, no mycoplasma-like inclusions were observed either in the F_1 or in the backcross males whose mothers were raised on the food that contained the antibiotic. However, the F_1 males treated with tylosin do show some disturbances in the symmetry of the axial filaments in the spermatids (Fig. 3). Males from the other treated series are not distinguishable (by electron microscopy) from the control males. The evidence is, therefore, consistent with the supposition that there is a causal relation between the presence of numerous mycoplasma-like bodies and the sterility of the hybrid males (*13*). The possibility cannot however be ruled out that the mycoplasma-like bodies are symbionts which thrive particularly in the cytoplasm derived from the degenerating spermatids in the hybrid males. On the other hand, the presence and the rate of multiplication of the mycoplasma may be genetically controlled; discord between the genotype of the host and the symbiont may allow the latter to multiply excessively and result in the sterility of the males. The enhancement of fertility in hybrid males whose mothers had been treated with antibiotics known to inhibit the multiplication of mycoplasma provides additional support for the hypothesis of causal relationship.

R. P. KERNAGHAN
Department of Biological Sciences, State University of New York, Stony Brook 11790

LEE EHRMAN
Rockefeller University, New York City 10021

References and Notes

1. Th. Dobzhansky and O. Pavlovsky, *Genetics* **55**, 141 (1967); L. Ehrman, *Quart. Rev. Biol.* **37**, 279 (1962).
2. L. Ehrman and D. L. Williamson, *Genetics* **62**, 193 (1969).
3. L. Ehrman, *Proc. Nat. Acad. Sci. U.S.* **58**, 195 (1967).
4. ———, *Mol. Gen. Genet.* **103**, 218 (1968).
5. R. P. Kernaghan and L. Ehrman, *Chromosoma* **29**, 291 (1970); *Abstr. Annu. Mtg. Amer. Soc. Cell Biol. 9th* (November 1969, Detroit, Mich.), p. 67a.
6. A. G. Newnham and H. P. Chu, *J. Hyg.* **63**, 1 (1965).
7. C. Friend, M. C. Patuleia, J. B. Nelson, *Proc. Soc. Exp. Biol. Med.* **121**, 1009 (1966).
8. R. F. Whitcomb and R. E. Davis, *Annu. Rev. Entomol.* **15**, 405 (1970); R. E. Davis, R. F. Whitcomb, R. L. Steere, *Science* **161**, 793 (1968).
9. S. Ohba, *Heredity* **22**, 169 (1967).
10. B. Spassky, *Drosophila Inf. Serv.* **17**, 67 (1943).
11. T. Ishiie, Y. Doi, K. Yora, H. Asuyama, *Annu. Phytopathol. Soc. Japan* **33**, 267 (1967); K. Maramorosch, E. Shikatu, R. R. Granados, *Trans. N.Y. Acad. Sci.* **30**, Ser. 2, 841 (1968).
12. L. Ehrman, *Science* **145**, 159 (1964); *Proc. Nat. Acad. Sci. U.S.* **49**, 155 (1963).
13. In *D. paulistorum* females, mycoplasma-like bodies are also found intracellularly in the cytoplasm of developing follicles in the ovaries.
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Tubules of Globoid Leukodystrophy: A Right-Handed Helix

Abstract. *Morphologic similarity between the cytoplasmic tubules of globoid leukodystrophy and Gaucher's disease (as demonstrated by thin sectioning, negative staining, and shadowing techniques) and their resemblance to negatively stained beef cerebroside are presented as evidence favoring the accumulation of cerebrosides in globoid cells. The tubules of globoid leukodystrophy have a 60-angstrom periodic banding similar to the tubules of Gaucher's disease. A right-handed helical twisting of the tubules is observed in both diseases.*

Information concerning the nature of the metabolic abnormality in any of the leukodystrophies may help to elucidate myelin metabolic pathways. The leukodystrophies are a heterogeneous group of genetically determined disorders of myelin metabolism. Each specific type has characteristic chemical alterations which are reflected in its morphology.

Globoid cell leukodystrophy (Krabbe's disease) is inherited as an autosomal recessive condition affecting infants usually in the first year of life. Progressive motor regression and mental deterioration become fatal within 1 or 2 years. Extensive demyelination is characteristically associated with the accumulation of a large number of globoid cells in the white matter of the brain.

The significance and origin of the globoid cells is not clear and has been the subject of extensive histochemical and biochemical studies (*1*). One hypothesis regarding the underlying enzymatic abnormality is that a deficiency of cerebroside sulfotransferase causes a block in the conjugation of cerebroside with sulfate to form sulfatide (*2*). This results in an increase in the ratio of cerebroside to sulfatides. More recently a deficiency of the enzyme galactose cerebrosidase has been reported in the tissues and leukocytes of patients with Krabbe's disease (*3*). This supports the idea that cerebroside is stored but not that sulfatide synthesis is blocked.

Three recent papers (*4, 5*) disclosed the presence of tubular structures in the cytoplasm of globoid cells. Two of them (*4*) described only straight to slightly arched polygonal structures