strongly than it does fusion. Diazepam will be important in evaluating mechanisms of myoblast fusion and myosin heavy chain synthesis, as well as in determining the relationship between contractility and myosin heavy chain synthesis.

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References and Notes

1. G. Zbinden and L. O. Randall, *Adv. Pharmacol.* **5**, 213 (1967); L. O. Randall, W. Schallek, L. H. Sternback, R. Y. Ning, in *Psychopharmacological Agents*, M. Gordon, Ed. (Academic Press, New York, 1974), vol. 3, p. 175.
2. H. Möhler and T. Okada, *Science* **198**, 849 (1977); R. F. Squires and C. Braestrup, *Nature (London)* **266**, 732 (1977).
3. H. P. Lubin and F. Robert, *Eur. Neurol.* **11**, 345 (1974); D. G. Berry, *Proc. Soc. Exp. Biol. Med.* **150**, 240 (1975).
4. H. Holtzer, *Excerpta Med.* **147**, 15 (1967); D. Fischman, in *The Structure and Function of Muscle*, G. H. Bourne, Ed. (Academic Press, New York, 1972), p. 75.
5. J. R. Coleman and A. W. Coleman, *J. Cell. Physiol. Suppl.* **72**, 19 (1968).
5a. B. Paterson and R. C. Strohman, *Dev. Biol.* **29**, 113 (1972).
6. C. R. Walker and B. W. Wilson, *Neuroscience* **1**, 191 (1976).
7. The concentrations of diazepam used in this study were higher than those normally found circulating in humans taking the drug. After a single dose of 10 to 20 mg, diazepam in the blood may reach 3.5 μM in 4 minutes [D. J. Greenblatt and R. I. Shader, *Benzodiazepines in Clinical Practice* (Raven, New York, 1974)]. However, diazepam crosses the placental barrier where it is metabolized only very slowly, if at all, by the developing fetus [A. P. Cole and D. N. Haley, *Arch. Dis. Child.* **50**, 741 (1975)]. Abuse of diazepam over a long period can result in diazepam serum concentrations that approach those used in this study [L. Foster and C. Frings, *Clin. Chem.* **16**, 177 (1970)].
8. C. P. Emerson and S. K. Beckner, *J. Mol. Biol.* **93**, 431 (1975); B. Vertel and D. Fischman, *Dev. Biol.* **37**, 63 (1976).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
10. P. Moss and R. C. Strohman, *Dev. Biol.* **48**, 431 (1976).
11. When 100 μM diazepam is added to pure fibroblast cultures, the cells appear more elongated and may have a slightly longer generation time. After growing for 3 days in diazepam-containing medium, the cells showed no effect on their protein content, although by 6 days, a 20 percent reduction in protein content was observed. This suggests that the slight reduction in total protein content seen in myogenic cultures may be a more general effect of the drug rather than solely reduction in myosin heavy chain content. However, the incorporation of [^{3}H]leucine into fibroblast protein during a 1-hour labeling period was unaffected by the drug.
12. H. Balzer, M. Makinose, W. Hasselbach, *Arch. Exp. Pathol. Pharmakol.* **260**, 444 (1968).
13. A. Shainberg, G. Yagil, D. Yaffe, *Exp. Cell Res.* **58**, 163 (1969); D. Yaffe and H. Dym, *Cold Spring Harbor Symp. Quant. Biol.* **37**, 543 (1972).
14. Supported by National Institutes of Health grant AM-13882 (R.C.S.), National Research Service award AM-05087 (E.B.), National Research Service award NS-05104 (C.R.W.), and a California Heart Association fellowship (C.R.W.). We thank Dr. Harlan Hill for providing diazepam.

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Pollen Abortion in T Cytoplasmic Male-Sterile Corn (*Zea mays*): A Suggested Mechanism

Abstract. *A rapid replication of mitochondria (20- to 40-fold increase) occurs between the precallose and tetrad stages in the tapetum of N and T corn* (Zea mays) *anthers, followed by mitochondrial, tapetal, and pollen breakdown in T anthers. It is suggested that the altered DNA in T mitochondria may malfunction under these stress conditions.*

With appropriate fixation and staining procedures, DNA fibrils can be demonstrated in plastids and mitochondria of higher plants. These fibrils form the basis of a limited nucleic acid system in these organelles, over and above the elaborate system found in the nucleus. Techniques such as the one described here (*1*) clearly show the DNA fibrils in both mitochondria and proplastids (Fig. 1, arrows).

Although limited in quantity, organellar DNA has the essential properties of nuclear DNA in being made up of linearly arranged base pairs and in having the capacities of self-replication and RNA transcription. It has become evident that this extranuclear DNA provides the basis of those characters that are not inherited in a Mendelian manner, and which for this reason have been termed "cytoplasmic."

The T-type male sterility in corn (*Zea mays*) is an example of cytoplasmic inheritance [reviewed in (*2*)]. It is not transmitted through the male, and crosses and backcrosses between male-sterile plants (T) and fertile maintainer plants (N) do not segregate. In a classic early work, Rhoades (*3*) showed that replacement of all nuclear chromosomes in a male-sterile line with chromosomes known to be free of sterility factors had no effect on the degree of sterility.

More recent work has shown that mitochondria from N and T plants react differently to toxins of *Helminthosporium maydis* Nisikado and Miyake, the agent of southern corn leaf blight (*4*). Levings and Pring (*5*) have shown that mitochondrial DNA's from N and T plants differ significantly after treatment with restriction endonucleases. We have observed that mitochondria in the tapetum and middle layers of plants with T cytoplasm become disorganized internally shortly after meiosis (*6*), and that this results in early tapetal vacuolation and degeneration. Since the tapetum serves as the source of nutrients for the young microspores, its early degeneration may well be related to pollen abortion. The T type of sterility in corn is thus clearly inherited cytoplasmically and would appear to be conditioned by an altered DNA in the mitochondria of plants with T cytoplasm.

Recent studies (*7*) on mitochondrial size and number in inbred lines of F_{44} N and T corn plants suggest an explanation for the mitochondrial breakdown observed in anthers with T cytoplasm. A statistically significant increase in numbers and decrease in size of mitochondria occurs in certain anther cells of both N and T plants (Table 1), but mitochondrial degeneration and microspore abortion occur only in plants with T cytoplasm. This mitochondrial replication is rapid (largely between precallose and tetrad stages, a period of some 3 days) and immediately precedes the observed degeneration in anthers with T cytoplasm.

During this period, sporogenous cells show a fourfold increase in number (as a result of the meiotic divisions), tapetal cells show a 1.3-fold average increase in number (as the result of occasional mitotic divisions), and the cells in both tissues show a four- to sixfold increase in volume. While these moderate increases are taking place in cell dimensions, mitochondrial numbers increase almost 20 times in sporogenous tissue and some 40 times in the tapetum, and individual mitochondrial volumes decrease 12 to 15 times (Table 1, ratio S_4/S_1). These changes are observed in the tapetum and sporogenous cells of both fertile and sterile anthers, but not in other anther tissues.

Other workers (*8*) have measured and counted mitochondria in plant cells, but we believe this is the first time that fertile and cytoplasmic male-sterile plants have been compared by such measurements. Also, the decrease in mitochondrial size and the rapid rate of mitochondrial replication observed in the present study, especially in the tapetum, appears to be unusual.

If organelles contain multiple and possibly a variable number of copies of the genome, as seems likely (*9*), we may question the effect of this rapid increase in number and decrease in size on mitochondrial DNA and protein mechanisms: This might well constitute a condition of

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Table 1. Mitochondrial size and number in sporogenous and tapetal cells of F_{44} corn anthers at six developmental stages. Figures are means of combined readings from three areas in each of two anthers at each stage from fertile (N) and sterile (T) plants. Complete calculation procedures are given elsewhere (*7*). There were no statistically significant differences between N and T cytoplasms and no interaction between cytoplasms and stages. The N and T cytoplasms, therefore, were combined in this table. Means followed by the same letter within a tissue do not differ at the 5 percent level according to Duncan's multiple range test.

Developmental stage	Mitochondrial number per unit volume (μm^3)		Mitochondrial volume (μm^3)		Cell volume (μm^3)		Mitochondrial number per cell	
	Sporogenous cells	Tapetum	Sporogenous cells	Tapetum	Sporogenous cells	Tapetum	Sporogenous cells	Tapetum*
S_1 precallose	0.544^{a}	0.255^{a}	0.202^{a}	0.288^{a}	12,274	846	6,677	216
S_2 central callose	0.887^{ab}	0.317^{a}	0.085^{b}	0.155^{b}	21,960	787	19,479	299
S_3 meiosis	2.032^{c}	0.902^{a}	0.010^{c}	0.043^{c}	33,189	1,962	67,440	2,478
S_4 tetrad	1.749^{bc}	2.179^{b}	0.016^{c}	0.015^{c}	71,780	3,099	125,543	8,779
S_5 young microspore	2.062^{c}	2.550^{b}	0.015^{c}	0.011^{c}	19,780	2,989	163,145†	9,909
S_6 intermediate microspore	1.410^{abc}	2.306^{b}	0.018^{c}	0.029^{c}	31,747	3,476	179,053†	9,619
Ratio S_4/S_1‡	3.2	8.5	−12.6	−15.2	5.8	3.7	18.8	40.6

*Values include limited cell division factor. †Includes the four microspores derived from the original pollen mother cell. ‡Stage S_4 was chosen for comparisons because it marks the beginning of mitochondrial breakdown in sterile anthers (*6*).

stress to which N and T mitochondria respond differently. Perhaps N mitochondrial DNA is able to replicate and facilitate the synthesis or utilization of essential gene products under the stress of rapid division in the anther; a slightly altered DNA in T mitochondria may not be able to do so. The reported differences in amino acid and isozyme content of N and T anthers [reviewed in (*2*)] may well be indications of such metabolic disturbances.

We also made a limited number of parallel investigations on organelle size and numbers in female reproductive tissues, where gamete abortion does not occur even in plants with T cytoplasm. The results were similar to those for anthers in that no differences between fertile and sterile plants were observed in size or number of mitochondria; they differed from the results with anthers, however, in that mitochondria in embryo sacs and nucellar cells were found to remain relatively constant in size and number during gametogenesis. This might suggest an effect of the rapid mitochondrial division observed in anthers: Gamete abortion does occur in anthers with T cytoplasm immediately after very rapid mitochondrial division; it does not occur in ovules of plants with T cytoplasm, in which there is no comparable mitochondrial multiplication.

Flavell (*10*) has proposed a model for cytoplasmic male sterility, based on a hypothetical substance regularly produced in and confined to the anther that inhibits the activity of mutated organelles. The demonstration of unusual mitochondrial replication in certain anther tissues (not in ovules), followed by mitochondrial breakdown in T anthers, may well provide the crucial differential for which Flavell proposed his "hypothetical substance(s)."

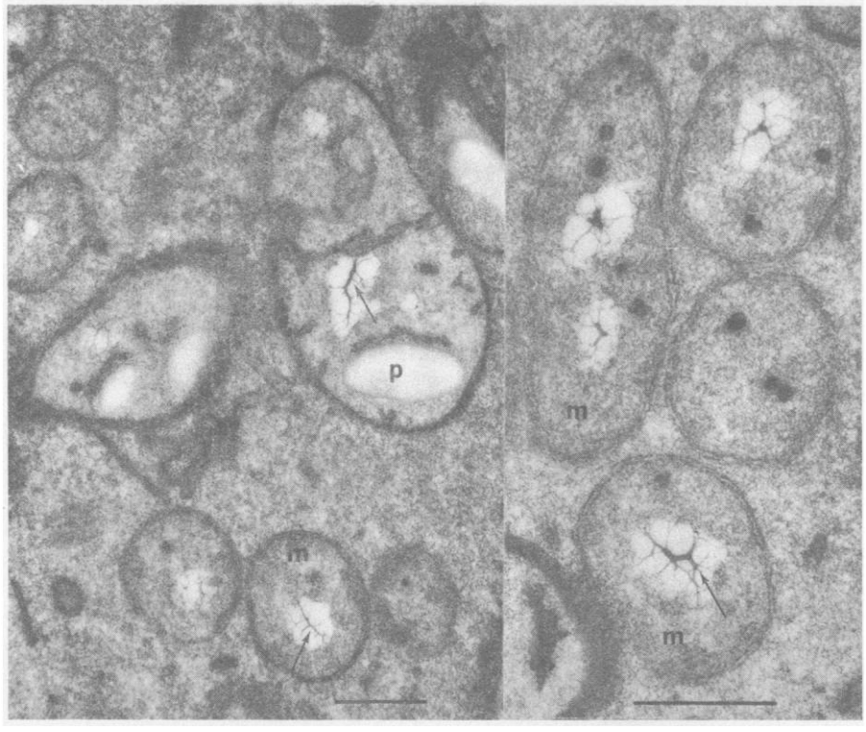

Fig. 1. Electron micrographs of DNA fibrils (arrows) in plastids (*p*) and mitochondria (*m*) from nucellar cells of T cytoplasmic male-sterile corn ovules. These fibrils provide the genetic information for characters inherited in a "cytoplasmic" manner. The tissue was fixed in $KMnO_4$ and then treated with uranyl nitrate (*1*). Scale bars are 0.5 μm. Organelles in ovules of fertile and male-sterile plants are not visibly different under the electron microscope.

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References and Notes

1. The tissue was fixed for 4 hours at 4°C in 0.5 percent aqueous $KMnO_4$, treated overnight in cold 1 percent uranyl nitrate in 70 percent acetone, passed through 100 percent acetone, and embedded in Spurr plastic. Sections were stained for 2 minutes in Reynold's lead citrate. This procedure does not preserve ribosomes or the cristae of mitochondria. Studies of mitochondrial size and numbers were made after glutaraldehyde-osmium fixation [M. J. Karnovsky, *J. Cell Biol.* **27**, 137A (1965)].
2. D. N. Duvick, *Adv. Genet.* **13**, 1 (1965); J. R. Edwardson, *Bot. Rev.* **36**, 341 (1970).
3. M. M. Rhoades, *J. Genet.* **27**, 71 (1933). The male-sterile line used by Rhoades has now been lost, but it was clearly the cytoplasmic type.
4. R. J. Miller and D. E. Koeppe, *Science* **173**, 67 (1971); P. A. Peterson, R. B. Flavell, D. H. P. Barratt, *Theor. Appl. Genet.* **45**, 309 (1975); H. C. Aldrich, V. E. Gracen, D. York, E. D. Earle, O. C. Yoder, *Tissue Cell* **9**, 167 (1977).
5. C. S. Levings, III, and D. R. Pring, *Science* **193**, 158 (1976).
6. H. E. Warmke and S. L. J. Lee, *J. Hered.* **68**, 213 (1977).
7. S. L. J. Lee, thesis, University of Florida (1976); ——— and H. E. Warmke, in preparation.
→ C. J. Avers, *Am. J. Bot.* **49**, 996 (1962); F. A. L. Clowes and B. E. Juniper, *J. Exp. Bot.* **15**, 622 (1963); B. E. Juniper and F. A. L. Clowes, *Na-*

ture (London) **208**, 864 (1965); L. Diers, *J. Cell Biol.* **28**, 527 (1966); C. Malone, D. E. Koeppe, R. J. Miller, *Plant Physiol.* **53**, 918 (1974).
9. M. M. K. Nass, *J. Mol. Biol.* **42**, 521 (1969); ——, *Science* **165**, 25 (1969); G. W. Grimes, H. R. Mahler, P. S. Perlman, *J. Cell Biol.* **61**, 565 (1974); S. D. Kung, *Annu. Rev. Plant Physiol.* **28**, 401 (1977).
10. R. B. Flavell, *Plant Sci. Lett.* **3**, 259 (1974).
11. Cooperative investigations of the Departments of Agronomy and Plant Pathology, University of Florida. Florida Agricultural Experiment Stations Journal Series No. 630. We thank V. Chew for help in statistical analysis.

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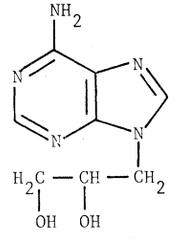
(*S*)-9-(2,3-Dihydroxypropyl)adenine: An Aliphatic Nucleoside Analog with Broad-Spectrum Antiviral Activity

Abstract. *(S)-9-(2,3-Dihydroxypropyl)adenine, a novel nucleoside analog, the sugar moiety of which is replaced by an aliphatic chain, inhibits the replication in vitro of several DNA and RNA viruses, including vaccinia, herpes simplex (types 1 and 2), measles, and vesicular stomatitis. It is also effective in vivo in reducing the mortality rate of mice inoculated intranasally with vesicular stomatitis virus.*

Besides interferon and its inducers, very few chemicals have been found to exhibit broad-spectrum antiviral activity. An example is ribavirin (1-β-D-ribofuranosyl - 1,2,4 - triazole - 3 - carboxamide), which was shown to inhibit the multiplication of a large variety of RNA and DNA viruses in both cell cultures and animals (*1*). Yet the antiviral action of ribavirin cannot be considered very specific, since it was found to inhibit DNA and RNA synthesis of the host cell at concentrations that coincided quite well with those at which it inhibited virus multiplication (*2*). Furthermore, ribavirin has been shown to exert both immunosuppressive and antitumor effects (*3*), to inhibit immune-complex glomerulonephritis in NZB/W mice (*4*), and to induce congenital anomalies when administered to pregnant hamsters (*5*).

We describe here the broad-spectrum antiviral activity of (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA], an aliphatic nucleoside analog, which inhibited the replication of a number of DNA and RNA viruses (vaccinia, herpes simplex, vesicular stomatitis, and measles) at concentrations at which cellular DNA and RNA synthesis were not affected.



(S)-DHPA

(*S*)-DHPA belongs to a class of novel nucleoside analogs, the sugar moieties of which are replaced by aliphatic chains. Although some aliphatic nucleoside analogs (for example, aristeromycin, eritadenine, and willardiin) occur in nature (*6*), such substances are not normally involved in either nucleic acid biosynthesis or catabolism. In their "D-glycero" [or (*S*)-enantiomeric] form, the 2,3-dihydroxypropyl derivatives of uracil and adenine imitate the conformation of the β-D-ribonucleosides (*6*): their circular dichroism spectra are similar to those of the natural ribonucleosides; their 2′,3′-cyclic phosphates are split by (some) ribonucleases, and their aminoacyl esters effectively inhibit the peptidyltransferase reaction (*7*).

Table 1. Antiviral activity of (*S*)-DHPA in cell culture. The (*S*)-DHPA was added 1 hour after the cells had been inoculated with ~ 100 times the virus dose needed to infect 50 percent of the cells (~ 100 $CCID_{50}$). The antiviral activity of (*S*)-DHPA is given as ID_{50}; that is, the dose inhibiting the cytopathogenic effect (CPE) of the virus by 50 percent. The CPE was recorded as soon as it reached completion in the untreated virus-infected cell cultures; in this way it was also possible to detect a delay in the CPE. Abbreviations: PRK, primary rabbit kidney; HSF, human skin fibroblast; GMK, green monkey kidney; Vero, a continuous line of green monkey kidney cells; and BHK, a continuous line of baby hamster kidney cells.

Virus	Cell culture	ID_{50} (μg/ml)
DNA viruses		
Vaccinia	PRK	10–20
Vaccinia	HSF	10–20
Herpes simplex 1 (strain KOS)	PRK	10
Herpes simplex 1 (strain KOS)	HSF	20
Herpes simplex 2 (strain 333)	PRK	4–10
Herpes simplex 2 (strain 333)	HSF	7–20
RNA viruses		
Vesicular stomatitis	PRK	7–10
Vesicular stomatitis	HSF	2– 7
Vesicular stomatitis	HeLa	>200
Poliovirus 1	HSF	>200
Poliovirus 1	HeLa	>200
Coxsackievirus B4	HeLa	>200
Coxsackievirus B4	Vero	>200
Measles	Vero	4–40
Newcastle disease	HSF	>200
Newcastle disease	GMK	>200
Sindbis	BHK	>200

The antiviral activity of (*S*)-DHPA was explored in several cell cultures with several viruses (see Table 1). After the cells were inoculated with one of the viruses, they were exposed to various concentrations of (*S*)-DHPA. For each virus-cell system, we determined the dose of (*S*)-DHPA required to suppress viral cytopathogenicity by 50 percent. As shown in Table 1, several viruses, including vaccinia, herpes simplex (types 1 and 2), measles, and vesicular stomatitis, were inhibited by (*S*)-DHPA. Others such as poliovirus, Coxsackievirus, and Sindbis were not.

That the inhibitory effects of (*S*)-DHPA on virus-induced cytopathogenicity actually reflected inhibition of virus multiplication was determined by measuring virus growth in human skin fibroblast cultures that had been inoculated with vesicular stomatitis virus (VSV) and subsequently exposed to (*S*)-DHPA (Fig. 1A). The (*S*)-DHPA (100 μg/ml) caused a dramatic decrease of virus titer. This reduction amounted to approximately 4 $\log_{10}$ for the virus yields measured at 24 and 48 hours after infection (Fig. 1A).

The potential activity of (*S*)-DHPA in vivo was assessed in mice inoculated intranasally with VSV. This experimental infection resembles certain natural infections in humans (such as poliomyelitis, rabies, and herpetic encephalitis) in that the virus spreads from a respiratory tract site (olfactory mucosa) through the (olfactory) nerves to the brain. The intranasal VSV model was employed previously to evaluate the prophylactic and therapeutic efficacy of interferon and its inducers (*8*). Repeated doses of (*S*)-DHPA (2 mg per mouse or ~ 135 mg/kg) injected intraperitoneally 1 hour and 1, 2, 3, and 4 days after VSV challenge brought about a significant increase in the final number of surviving mice (Fig. 1B): 67 percent for the (*S*)-DHPA–treated mice compared to 37.5 percent for the control group ($P < .05$, chi-square test with Yates' correction). When the numbers of survivors were compared 9 days after infection, the difference between the (*S*)-DHPA group and the control group was significant at $P < .005$. Repeated doses of (*S*)-DHPA at 0.08 mg per mouse (~ 5.4 mg/kg) did not confer protection, whereas repeated doses at 0.4 mg per mouse (~ 27 mg/kg) gave slight protection (the final numbers of surviving mice were 55 percent for the treated group and 37.5 percent for the

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