to 34 during postembryonic development, the intestinal cells themselves apparently do not divide (*9*).

The x-irradiation could also have affected the development of the animal. For instance, x-ray–induced death of cells followed by cell rearrangements could affect the size and shape of the fluorescent patches, and Sulston (*10*) has found that intestinal cells can functionally regulate for the loss of neighboring intestinal cells by expansion, without undergoing compensatory divisions. However, all the fluorescent patches we observed fit the known E cell lineage, indicating such artifacts apparently did not occur under our conditions.

About 70 percent of the mosaic animals were sterile. This infertility might have been due to the x-irradiation or to the intense ultraviolet light to which the animals were exposed when they were examined for fluorescence. However, the remaining 30 percent that were fertile gave rise to dumpy, fluorescent, and wild-type animals in about Mendelian proportions (1:1:2), suggesting that the germ cells of the mosaic animals were heterozygous for the *flu-3* and the *dpy* markers. The fluorescent patches indicate a somatic segregation of the *flu* allele in the intestinal lineage and autonomous expression of the mutant *flu-3* character. The mosaics could arise by a variety of mechanisms, such as chromosome elimination, somatic mutation, chromosome inactivation, or somatic recombination.

Our experiments show that a fluorescent mutant of *C. elegans* can be used in detecting genetic mosaics in the intestine of the nematode. The *flu-3* mutation is expressed only in the intestinal tissue (*6*). There are also mutations in *C. elegans* that are specific for other tissues. They affect, for example, the body muscle structure as observed by polarized light microscopy (*11*) or nervous tissue as observed by histochemical staining for acetylcholine esterase (*12*). If such mutants are cell-autonomous, it may be possible to detect mosaic cells in a normal background.

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Polyamine Metabolism: A Potential Therapeutic Target in Trypanosomes

Abstract. *α-Difluoromethylornithine (RMI 71,782), a specific irreversible inhibitor of the first step in polyamine biosynthesis, that is, the formation of putrescine from ornithine by ornithine decarboxylase, cures mice infected with a virulent, rodent-passaged strain of* Trypanosoma brucei brucei. *This parasite is closely related to the trypanosomes that cause human sleeping sickness. The drug, which is remarkably nontoxic, was effective when administered in drinking water or by intubation. The ability of the compound to inhibit ornithine decarboxylase in vitro was demonstrated by the reduced amounts of putrescine synthesized from tritiated ornithine in* Trypanosoma brucei *suspensions. These observations direct attention to polyamine metabolism as a target for chemotherapy of parasitic diseases.*

Trypanosoma brucei brucei causes trypanosomiasis (nagana) in cattle, and *T. b. rhodesiense* and *T. b. gambiense* cause sleeping sickness in humans. Collectively these diseases severely hinder the economic and social development of sub-Saharan Africa. The drugs now used for these diseases are highly toxic (*1*) and induce drug resistance, and no new drug has been introduced in the last 25 years (*2*). We have found that a highly specific inhibitor of polyamine biosynthesis, α-difluoromethylornithine (DFMO, RMI 71,782), blocks multiplication of the

Table 1. Effects of DFMO on *T. b. brucei* infection in mice. Groups of five animals (20 to 25 g) were inoculated with *T. b. brucei* (EATRO 110 isolate; 5×10^5 organisms per animal). Treatment was begun 24 hours after infection. Results are expressed as average survival (in days) beyond death of control based on an average control survival of 5 days. Berenil (diminazene aceturate) is included as a control trypanocide. The drinking water containing DFMO or α-methylornithine was constantly available.

Drug and treatment regimen	Total dose* (mg)	Average survival (days)
None	0	0
α-DFMO in drinking water		
2.0 percent for 6 days	600	> 30†
2.0 percent for 3 days	300	> 30
1.0 percent for 6 days	300	> 30
1.0 percent for 3 days	150	> 30
0.5 percent for 3 days	75	28.6
0.1 percent for 3 days	15	2
α-DFMO by intubation		
300 mg/kg daily for 3 days	22.5	26.3
150 mg/kg daily for 3 days	11.3	22.8
75 mg/kg daily for 3 days	5.6	19.2
50 mg/kg daily for 3 days	3.8	0
α-methylornithine in drinking water		
2.0 percent for 3 days	300	0
Berenil injected intraperitoneally		
2.5 mg/kg daily for 3 days	0.2	> 30

*The dose administered in drinking water was calculated on the basis of a daily intake of 5 ml of water per 25-g mouse per day. †Considered curative. Animals survived more than 1 month beyond controls; blood smears were negative for parasites after 1 month. Uninfected mice inoculated with brain suspensions from cured animals remained free of disease for more than 30 days.

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parasite *T. b. brucei* in mice and eliminates the infection.

Polyamines are low-molecular-weight compounds found in all cells, where they are thought to participate in nucleic acid and protein synthesis and to stabilize certain macromolecules (*3–5*). The common polyamines are putrescine (1,4-diaminobutane), spermidine, and spermine. The last two molecules are synthesized from putrescine by addition of aminopropyl groups derived from *S*-adenosyl-L-methionine.

The rate-controlling step in mammals for formation of polyamines appears to be that catalyzed by ornithine decarboxylase (ODC) (E.C. 4.1.1.17), an enzyme that is extremely susceptible to external inducers and has a high turnover rate (*6, 7*). Because polyamine synthesis and ODC induction are associated with onset of growth in all prokaryotic and eukaryotic organisms thus far studied (*4, 5, 8*), ODC is a logical target for chemotherapy of disease states characterized by rapid cell proliferation. The ability of DFMO to inhibit ODC and growth in various mammalian cells and organs has been reported (*9–12*). Such reports, coupled with the remarkably low toxicity of DFMO (*9*), suggested trial of the drug in trypanosome infections in the mouse.

Management of infections in mice produced by the pleomorphic EATRO 110 (East African Trypanosome Research Organization) isolate of *T. b. brucei*, maintained by rat passage, has been detailed (*13, 14*). In general, we tested drug activity against established infections in which animals were infected with 5×10^5 parasites 24 hours before testing. Animals so infected die about 5 days after inoculation. The effects of DFMO in this model, administered by different routes and in varying dosages for periods of 3 to 6 days, are summarized in Table 1. Groups of animals receiving the drug as a 1 or 2 percent solution in their drinking water were totally cured of infection, as indicated by survival times greater than 30 days beyond that of untreated control animals. Animals cured of infection remained parasite-free more than 30 days after deaths of controls; most animals were held more than 2 months without relapse. Parasites disappeared from blood 5 days after treatment was begun, and blood smears taken 30 days after death of controls were negative. Healthy mice injected with brain suspensions [sometimes the site of cryptic trypanosome infection (*15*)] from cured animals failed to develop parasitemia.

One dose of DFMO per day for 3 days administered by intubation was generally less effective than the DFMO given in drinking water, although some prolongation of survival was noted with doses of 75 to 300 mg/kg. These results were not surprising since it is known that the drug is rapidly cleared in rodents and frequent dosing (as when the drug is administered in drinking water) is required for optimal biochemical effects (*12*). The total amount of drug ingested when DFMO was given as a 1 or 2 percent solution in the drinking water was significantly higher than any of the total doses given by intubation. The mice showed no toxic effects even with the highest total doses of DFMO, as was observed previously (*9, 12*). The relatively weak ODC competitive inhibitor, α-methylornithine, effective in some instances in reducing intracellular polyamine concentrations in vitro (*16*), did not prolong the life of infected mice. Berenil (diminazene aceturate) a routinely used trypanocide, was included as a positive control (Table 1).

Table 2. Effects of DFMO on synthesis of putrescine from [3-^{3}H]ornithine in bloodstream and culture forms of *T. b. brucei*. Cells were incubated for 1 hour in 3-ml portions in a rotary shaker (100 rev/min). Blood forms from a 72-hour infection in a rat were incubated at 37°C (2×10^8 parasites per milliliter) in RPMI 1640 containing 25 m*M* Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid) and 20 percent heat-inactivated fetal bovine serum (*26*). Culture forms were from a 1-week culture in Medium 199 containing 20 percent fetal bovine serum (25°C; 5×10^7 parasites per milliliter). Cell extracts were complexed to form dansyl derivatives of polyamines. These were chromatographed and the spots containing putrescine were scraped into scintillation vials for counting. For complete details concerning cell isolation and polyamine estimation see (*17*). Results are expressed as percentage inhibition of putrescine synthesis.

Cell type	Concentration of DFMO (m*M*)*					
	0.5	0.25	0.1	0.075	0.05	0.025
Culture	94		83		38	24
Bloodstream		82	73	74	65	43

*Results are the average of two or more experiments. Averages of specific activities in simultaneously run control samples with no DFMO present, when expressed as counts per minute per milligram of protein per hour, were 3.15×10^5 for culture forms and 3.4×10^5 for bloodstream forms.

That DFMO acts in trypanosomes by blocking polyamine biosynthesis is supported by studies in vitro of putrescine synthesis from L-[3-^{3}H]ornithine (New England Nuclear). When DFMO was added to the medium of intact trypanosomes in suspension according to a method described previously (*17*), putrescine biosynthesis was blocked (Table 2). We also found that 10 μ*M* to 25 μ*M* concentrations of DFMO blocked putrescine formation by more than 50 percent in crude *T. b. brucei* extracts. Endogenous biosynthesis via decarboxylation of ornithine seems to be the only manner in which *T. b. brucei* can obtain putrescine since the organism's ability to assimilate exogenous preformed radioactive putrescine, spermidine, and spermine (as, for example, in mammalian blood) was severely restricted (*17*).

Polyamine metabolism as a target for chemotherapy of parasites has been discussed (*18*). The value of this approach is supported by the demonstration of drug-polyamine interactions in trypanosomatids. Polyamine uptake and synthesis coupled with growth inhibition was detected in *Leishmania* sp. promastigotes treated with the trypanocides pentamidine and ethidium (*19*). Spermidine and spermine blocked the therapeutic effects of imidocarb and amicarbalide in *T. b. brucei* (*14*). Polyamines may have special significance for the metabolism and hence for chemotherapy of African trypanosomes, since bloodstream trypomastigotes depend completely on an α-glycerophosphate shuttle for the terminal step in respiration (*20*). A critical enzyme of this pathway, nicotinamide adenine dinucleotide (NAD^+)-linked α-glycerophosphate dehydrogenase, requires polyamines for full activity (*21, 22*). Cationic trypanocides such as Antrycide (quinapyramine) and ethidium interfere with the stimulation of this enzyme (*23*). Furthermore, we have recently isolated and partly purified two types of DNA polymerases from *T. b. brucei* and one of these enzymes, which differs in its catalytic properties from known mammalian DNA polymerases, is activated by polyamines (*24*). Since DFMO treatment causes a gradual reduction in parasitemia before final clearance of the blood after 5 days, this action seems more consistent with a mechanism of action involving blockade of cell replication rather than cellular toxicity.

Murine *T. b. rhodesiense* infections have also recently been cured by administration of 2 percent DFMO in drinking water (*25*). The nontoxic nature of DFMO and the success of oral administration—both unprecedented in trypanosomal chemotherapy—underscore the

potential utility of this approach. The role of polyamines in trypanosomes and their probable dependence on ODC as sole putrescine source emphasize their likely vulnerability to specific inhibitors of polyamine biosynthesis. Inhibitors of ODC may be particularly useful both as sole agents and in combination with currently available trypanocidal compounds.

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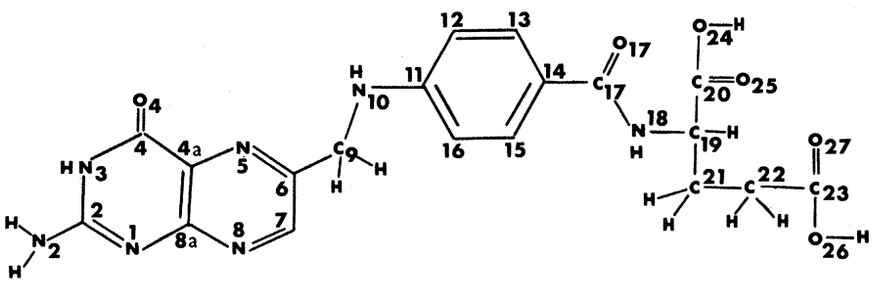
Folic Acid: Crystal Structure and Implications for Enzyme Binding

Abstract. *The crystal and molecular structure of folic acid dihydrate has been determined by x-ray diffraction. Folic acid is in an extended conformation with the pteridine ring in the keto form. The C(4) oxygen and N(10) atoms are on the same side of the molecule, hydrogen-bonded to the same water. This conformation has the pteridine rotated approximately 180° away from the orientation of the pteridine ring of methotrexate bound to dihydrofolate reductase. The folic acid pteridine and phenyl rings interact in a stacking manner which is suggestive of the type of associations these groups could form in a complex of folate, dihydrofolate reductase, and reduced nicotinamide adenine dinucleotide phosphate.*

The B vitamin folic acid is a necessary cell growth factor; the reduced form of the vitamin, 5,6,7,8-tetrahydrofolate, is an essential cofactor in a large number of enzyme reactions involving one-carbon transfers, including the synthesis of thymidylate from deoxyuridylate. Since a lack of thymidylate stops DNA synthesis and cell division, blockade of tetrahydrofolate formation generally leads to cell death. In mammalian cells folic acid is reduced first to 7,8-dihydrofolate and then to tetrahydrofolate, both steps being catalyzed by the enzyme dihydrofolate reductase (DHFR). This system has been the focus of a great deal of



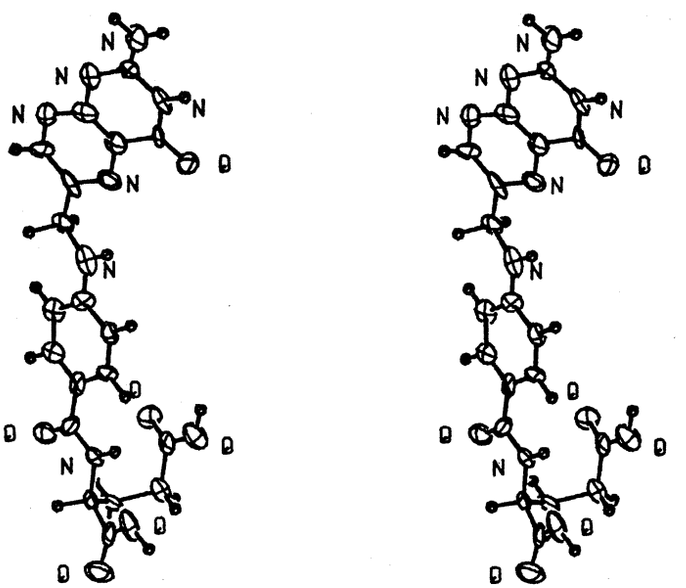


Fig. 1 (above). Folic acid chemical structure and atom numbering scheme. Fig. 2 (right). Stereoscopic drawing of the folic acid molecular structure.