upon long-term exposure (*11*). We know of no direct evidence for the carcinogenicity of HZ in humans (*12*), although specific epidemiologic studies are not available (*13*). Both HZ and its metabolites are rapidly excreted, primarily in the urine (*14*). An important enzyme in the metabolism of HZ is *N*-acetyltransferase (NAT) (*15*), which has a polymorphic distribution in humans (*16*), individuals being either rapid or slow acetylators. The development of HZ toxicity (*17*) seems to be more frequent in slow acetylators (*18*). Therefore, we examined the relationship between the genotoxicity of HZ and NAT activity in hepatocytes isolated from rats and from rabbits, a species exhibiting an acetylator polymorphism like that of humans (*19*). In rat or slow acetylator rabbit hepatocytes, the half-life of sulfamethazine, the substrate for NAT used in determining acetylator phenotype, was similar and over 15 times that of rabbit hepatocytes from a rapid acetylator (*20*). We found that HZ was genotoxic in rabbit hepatocytes with a slow rate of acetylation but not in those with a rapid rate of acetylation (*20*), although all hepatocyte preparations responded to the positive control aromatic amine carcinogen 2-aminofluorene. These observations suggest that rapid acetylation may diminish the genotoxic effect of HZ. Thus, slow acetylators in the human population may be more susceptible to the genotoxic effects of these hydrazine drugs. We conclude that HZ and DHZ are potential human carcinogens and that evidence for or against their actual carcinogenicity must be vigorously pursued.

GARY M. WILLIAMS
Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595
GUY MAZUE
Service de Toxicologie, Centre de Recherches Clin-Midy, 34082, Montpellier, France
CHARLENE A. MCQUEEN
TOMIKO SHIMADA
Naylor Dana Institute for Disease Prevention

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20. In collaboration with Dr. W. Weber of the University of Michigan, we compared the NAT activity and genotoxicity of HZ in hepatocytes from rapid and slow acetylator rabbits to that in rat hepatocytes. For the HPC/DNA repair test, we followed the procedures of Williams (*5*), using $10^{-3}M$ HZ. Hepatocytes were freshly isolated from adult Fisher F344 rats or New Zealand white rabbits and exposed to the test compound as described in Table 1. A positive result of 24.8 ± 5.5 grains per nucleus was obtained in rat hepatocytes. The slow acetylator rabbit cells gave a value of 17.7 ± 4.0, whereas the rapid acetylator rabbit hepatocytes at the highest nontoxic dose showed only a borderline result of 4.8 ± 2.4. For NAT activity, hepatocytes were incubated with $10^{-4}M$ sulfamethazine (SMZ) per milliliter of medium. Since the decrease in the SMZ concentration is proportional to the increase in *N*-acetyl SMZ, one can determine the rate of acetylation by measuring the disappearance of SMZ from the medium [A. C. Bratton and E. K. Marshall, *J. Biol. Chem.* **218**, 537 (1939); D. J. Hearse and W. W. Weber, *Biochem J.* **132**, 519 (1973)]. The logarithm of the SMZ concentration was plotted against time and the half-life determined by regression analysis. The half-life of SMZ was 50 hours for rat hepatocytes, 49 hours for hepatocytes from a slow acetylator rabbit, and only 3 hours for hepatocytes from a rapid acetylator rabbit.
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Genetic Mosaics of *Caenorhabditis elegans*: A Tissue-Specific Fluorescent Mutant

Abstract. *Genetic mosaics can be generated by x-irradiation in the simple nematode* Caenorhabditis elegans. *A mutation in the gene* flu-3 *alters the characteristic autofluorescence of intestinal cells under ultraviolet light and can be used as a cell- and tissue-specific marker. Embryos heterozygous for* flu-3 *give rise to adults with patches of these altered intestinal cells. The previously established intestinal cell lineage in* Caenorhabditis elegans *and the distribution and sizes of the fluorescent patches are consistent with a somatic segregation of the* flu-3 *allele.*

Genetic mosaics are useful for studying cell lineage and cellular interaction in development (*1*). Several interesting problems in development such as the construction of fate maps, estimation of number of primordial cells, analysis of cell differentiation, pattern formation, sex-determination, and behavior have been studied by means of mosaics and chimeras in *Drosophila* and mouse (*2*). The free-living soil nematode *Caenorhabditis elegans*, with its simple cellular anatomy, is particularly amenable to genetic manipulation (*3*, *4*). Here we demonstrate the use of a fluorescent marker to detect genetic mosaics in the intestinal tissue of *C. elegans*. A mutation in the gene *flu-3* gives rise to an altered intestinal autofluorescence which is both purple and more intense than that of the wild type. The mutant fluorescent phenotype is fully recessive with respect to the wild type (*5*, *6*).

Developing embryos heterozygous for the fluorescent marker were exposed to x-irradiation in the hope that it would enhance the frequency of mosaics, since we did not observe any spontaneous occurrence of mosaics among about 20,000 unirradiated heterozygous *flu-3* animals.

Males homozygous for the recessive fluorescent marker *flu-3 (t301)II* were crossed with dumpy hermaphrodites *dpy-5(e61)I* so that we would be able to distinguish heterozygous outcross progeny from any hermaphrodite progeny produced by self-fertilization (*3*). The non dumpy progeny resulting from cross-fertilization are heterozygous for both the morphological *dpy* marker and for the fluorescent marker *flu-3*. They are easily distinguishable from the dumpy

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Table 1. Frequency and size of the mosaic fluorescent patches with respect to the age of the embryo when irradiated.

Age of embryos when x-irradiated	Number of heterozygotes screened	Number of *flu-3* patches observed	Frequency of the *flu-3* patches observed (%)	Type of patch*			
				WI	AHI	PHI	SmI
Irradiated inside the hermaphrodite	97,212	62	0.06	18	25	19	
0 to 2 hours after egg was laid	25,544	4	0.02				4
2 to 4 hours after egg was laid	4,416	2	0.05				2
4 to 6 hours after egg was laid	3,120	1	0.03				1
Young larvae (stages 1 or 2)	4,200						

*Abbreviations: WI, whole-intestine mosaic; AHI, anterior half-intestine mosaic; PHI, posterior half-intestine mosaic; SmI, small fluorescent patch in the intestine. Each animal was removed from the petri plate after examination to avoid duplicate scoring of the heterozygotes.

progeny resulting from self-fertilization. The mated dumpy hermaphrodites, containing heterozygous embryos, were x-irradiated with 2000 rads at a dose rate of about 200 rad/min (Andrex x-ray machine, Model 4200) in a petri plate on the surface of NG agar covered with a thin lawn of *Escherichia coli* OP50 (*3*). After x-irradiation, the animals were transferred individually to fresh petri plates and allowed to lay eggs. These plates were incubated at 25°C for 2 to 3 days, until the irradiated embryos matured into adult animals. The non dumpy animals (heterozygotes) were scored under ultraviolet light (300 to 400 nm) for the appearance of fluorescent patches in the intestine.

The animals were x-irradiated at different stages of development in the hope that different patterns of mosaicism would be induced. Embryos in the early phase of development (up to about the 50-cell stage) were irradiated while still inside the mother, and late embryos (between about the 50-cell stage and hatching) after the eggs had been laid. Three different types of fluorescent patches were observed (Fig. 1). Their quantitative distribution varied with the age of the embryos at the time of x-irradiation (Table 1). Out of 97,212 heterozygotes, 62 animals that were irradiated during the early stages of embryonic development (from zygote formation to the time when the egg is laid) showed fluorescent patches. Of these animals, 18 (about 30 percent) were so-called "whole-intestine" mosaics; the fluorescent patch covered the entire intestine, so that the intestinal fluorescence of these heterozygous animals resembled that of homozygous animals but was more intense than wild type. The remaining 44 animals were "half-intestine" mosaics. In 25 (40 percent) of these, the anterior half of the intestine was fluorescent, whereas in the other 19 (30 percent), the posterior half was fluorescent. In this category there was no animal with a small fluorescent patch.

No whole-intestine nor half-intestine mosaics were observed when eggs were x-irradiated from 0 to 2 hours after they had been laid. However, four animals with small fluorescent patches were observed; two had patches in the most anterior region of the intestine and the other two in the most posterior region. Similarly, eggs that were irradiated 2 to 4 hours after being laid produced two animals with small fluorescent patches in the most anterior region of the intestine. Again, in this later category there was no animal with a whole-intestine or half-intestine mosaic.

Except in one animal, the mosaic patches continued to show the *flu-3* fluorescence during the life of each animal. In the one exception, the animal showed a very small patch of fluorescence (presumably involving only 1 or 2 cells) in the most posterior region of the intestine which gradually decreased in intensity and in 2 days was reduced to the intensity of the wild type.

To establish the presence of the *flu-3* gene in the fluorescent mosaics, x-irradiated control heterozygous embryos (not containing the mutant *flu-3*) were generated by crossing wild-type males with dumpy hermaphrodites. About 13,000 animals were screened for mosaicism, but none was found. Thus, under the conditions we used, there does not seem to be any developmental anomaly which mimics the *flu-3* mutant phenotype.

The observed mosaic patches fit the lineage of the embryonic E cell (the precursor of the intestine) (*7*). The first division of the E cell generates an anterior (E.a) and a posterior (E.p) daughter, the precursors for the anterior and posterior intestine, respectively. These continue to divide to give eight descendants each. The observed patches in the anterior or posterior halves of the intestine are consistent with this lineage. Two of the remaining four embryonic divisions (to give 20 intestinal cells at hatching) occur at the anterior end of the intestine and the other two at the posterior end (*8*). The patches at the anterior or posterior ends of the intestine are also consistent with this lineage. When young larvae (stages 1 and 2) were irradiated, no fluorescent patches were observed. This is as expected because, although the number of intestinal nuclei increases from 20

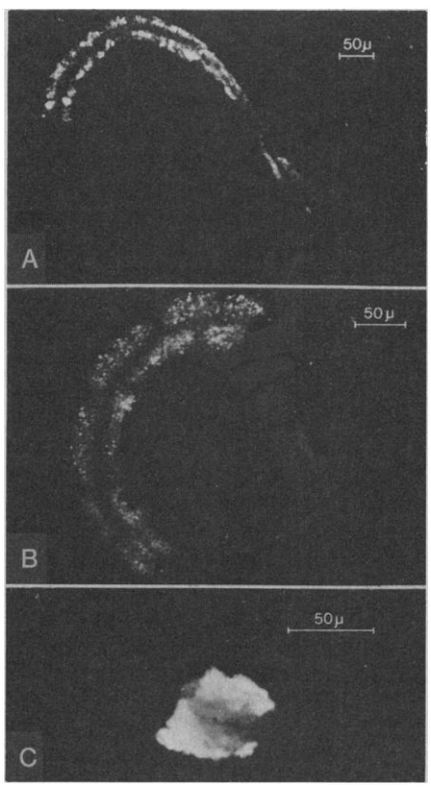

Fig. 1. Fluorescent patches induced by x-irradiating embryos heterozygous for the *flu-3* marker. (A) Whole-intestine mosaic. The distribution of the autofluorescence is identical to that in an animal homozygous for the *flu-3* mutation. (B) Half-intestine mosaic. The anterior half shows the characteristic *flu-3* autofluorescence while the posterior half of the intestine shows the wild-type fluorescence. (C) A small patch of intestinal mosaicism. Such small patches were always restricted to either the anterior most cells as shown here or to the posterior most cells of the intestine. The exact number of cells forming the fluorescent patches has not been determined. These photomicrographs were made in epifluorescence under ultraviolet light in the range of 300 to 400 nm, with a Zeiss Universal Photomicroscope equipped with an exciting filter (UG1/UG5) and a barrier filter No. 487709. The original color photomicrographs were on Kodachrome (160 ASA).

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.

SHAHID S. SIDDIQUI*
P. BABU
Tata Institute of Fundamental Research, Bombay 400 005, India

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* Present address: Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, 3400 Göttingen, Federal Republic of Germany.

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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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