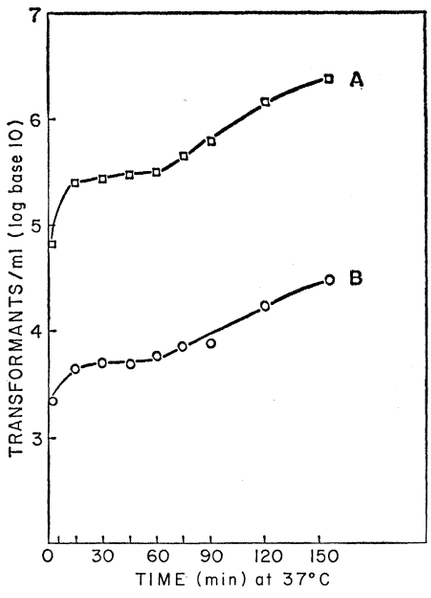

Fig. 2. Comparison of the replication of nitrous acid–induced transformants and "normal" transformants. *Haemophilus influenzae* competent cells (1×10^8/ml) were exposed to (*A*) DNA (0.2 μg/ml) from streptomycin-resistant cells and (*B*) nitrous acid–treated DNA (0.5 μg/ml) from streptomycin-sensitive cells. Samples were taken, diluted, and plated with antibiotic agar and incubated for 2 hours at 37°C. Then agar containing streptomycin was added to select for streptomycin-resistant cells. The final concentration of streptomycin was 8 μg/ml. Background has been subtracted.

allowing the transformed cells to grow and measuring the increase in the number of transformants as a function of time. After the samples were taken and plated, time was allowed for all the transformants to express fully before they were challenged with the antibiotic. Transformants from both the untreated streptomycin-marked DNA and nitrous acid–induced mutants began to replicate after about 60 minutes (Fig. 2). Therefore, no additional time was required for the nitrous acid–induced characteristic to be replicated. The same results were found with the antibiotic markers novobiocin (0.5 μg/ml) and dalacin (8 μg/ml). In addition to the time of replication, the rate of replication was the same as the rate of growth on the whole cell population, that is, a generation time of about 30 minutes during the logarithmic phase of growth.

Thus, genetic markers are produced directly on the DNA in vitro during exposure to nitrous acid. During transformation these markers closely resemble the behavior of "normal" genetic markers. The expression curves and the time and rates of multiplication of transformants of "normal" and nitrous acid–induced markers are essentially identical. This evidence places certain restrictions on the base-transition hypothesis of nitrous acid mutagenesis in the present system. If we assume that transitions take place, these transitions must not delay the incorporation process and the replication of the incorporated entity; the transforming molecule containing deaminated bases recombines with the recipient DNA and produces a marker which is functional and normal in subsequent replications.

One possibility is that the mutations observed here are all deaminations of cytosine to uracil which, in turn, may behave like thymine with regard to its pairing function and its ability to transcribe. On the other hand, a deamination of adenine, resulting in hypoxanthine, would probably not be consistent with the results reported since hypoxanthine would be expected to function more like adenine than guanine. If such mutations occur in the system we have tested, they must be selected against.

It seems unlikely that the nitrous acid mutations represent deletions produced in solution, since these deletions would break denatured DNA and greatly lower the efficiency of renaturation. In addition, mutations to low levels of streptomycin, which have been tested, are revertible (*8*).

The finding that mutations or the change leading to mutations occur on heat-denatured DNA, which is presumably single stranded, and the fact that the onset and rate of replication of mutants and transformants are the same, lead to the conclusion that only one of the two strands of DNA is sufficient in the transformation process. A more extensive series of analogous experiments with heat-denatured and renatured DNA's lead to the same conclusion (*9*). These results agree with similar conclusions derived in completely different ways (*10*).

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Tryptamine Oxidation by Extracts of Pea Seedlings: Effect of Growth Retardant β-Hydroxyethylhydrazine

Abstract. *β-Hydroxyethylhydrazine forces flowering of pineapple plants, possibly by reducing auxin concentration. A specific and sensitive assay showed that β-hydroxyethylhydrazine (3.3×10^{-7}M) inhibits noncompetitively and almost completely the oxidation of tryptamine to indoleacetaldehyde by extracts of pea-seedling* (Pisum sativum *L. var. Alaska). The relative rate of oxidation of putrescine to Δ^1-pyrroline compared to that of tryptamine by these extracts and the inhibition of both reactions by β-hydroxyethylhydrazine suggest that diamine oxidase is inhibited. β-Hydroxyethylhydrazine is a potent inhibitor in vivo of animal diamine oxidase.*

In 1955 Gowing and Leeper (*1*) found that β-hydroxyethylhydrazine (BOH) induces flowering in pineapple. The first action of this compound was retardation of stem elongation (*2*); then flowering was initiated. It has been suggested that this compound induces flowering by reducing the concentration of native auxin (*3*).

Although the pathways for biosynthesis of indoleacetic acid are not clearly established, one possible route in certain plants, such as pineapple, is by decarboxylation of tryptophan to tryptamine, which in turn may be oxidized to indoleacetaldehyde by diamine oxidase (*4*). Clarke and Mann (*5*) have found that tryptamine is converted to indoleacetaldehyde by diamine oxidase from pea seedlings.

The inhibitory effects of hydrazines on animal amine oxidases have been studied, yet little is known concerning the effects of these inhibitors on plant amine oxidase. However, Hill and Mann (*6*) reported changes in the absorption spectrum of purified pea-seedling diamine oxidase after addition of hydra-

Table 1. β-Hydroxyethylhydrazine (BOH) inhibition of indoleacetaldehyde-2-C^{14} formation from tryptamine-2-C^{14} by pea-seedling extracts. The reaction mixtures contained tryptamine-2-C^{14} (208, 200 DPM) 50 mμmole in 0.1 ml borate-phosphate buffer; BOH in 0.1 ml buffer, enzyme preparation 0.1 ml, and buffer to make total volume of 0.4 ml. The mixtures were incubated immediately after the addition of the enzyme preparation for 30 minutes at 26.5° ± 0.5°C. The disintegrations per minute (DPM) are expressed as mean ± average deviation of duplicate samples. Data are corrected for blank values (85 to 110 DPM) obtained by incubating tryptamine-2-C^{14} with boiled enzyme.

Concn. of BOH (molar)	Indoleacetaldehyde-2-C^{14}		Inhibition (%)
	Extracted (DPM)	Formed (mμmole)	
0	3341 ± 99	0.80 ± .02	0
1.0×10^{-9}	3392 ± 56	.81 ± .01	−2
1.0×10^{-8}	3280 ± 198	.79 ± .05	2
3.3×10^{-8}	3093 ± 18	.74 ± .00	8
1.0×10^{-7}	2245 ± 66	.54 ± .02	33
3.3×10^{-7}	794 ± 2	.19 ± .00	76
1.0×10^{-6}	280 ± 18	.07 ± .01	92
1.0×10^{-5}	34 ± 8	.008 ± .002	99

zine. They concluded that Cu^{++} and possibly pyridoxal phosphate are prosthetic groups for this enzyme. Werle *et al.* (*7*) reported that isopropylhydrazine is a potent, irreversible inhibitor of plant amine oxidase.

This work provides evidence that BOH is an irreversible inhibitor of the oxidation of tryptamine to indoleacetaldehyde by diamine oxidase in pea-seedling homogenates. Etiolated pea (*Pisum sativum* L. var. Alaska) epicotyl and plumule tissue was used to study diamine oxidase activity. After 7 to 9 days growth at 25° to 27°C in the dark, the seedlings were cut 10 to 15 mm below the plumule. The epicotyls (150 mg fresh wt.) were homogenized in 10.0 ml of a borate-phosphate buffer (0.033*M* each) at *p*H 8.1 (*5*) and the cellular debris was removed by centrifugation. The supernatant was diluted fivefold with additional buffer and used immediately in the enzyme assay. The diamine oxidase activity was measured essentially by the method described by Wurtman and Axelrod (*8*) for animal monoamine oxidase. In this specific and very sensitive assay, the amine oxidase is measured by the oxidation of tryptamine-2-C^{14} (New England Nuclear) to indoleacetaldehyde-2-C^{14}. The reaction was stopped by addition of HCl and the indoleacetaldehyde-2-C^{14} formed was extracted in toluene. After centrifugation, the radioactivity in a sample of the toluene extract was counted in a liquid scintillation spectrometer. Less than 0.05 percent of the remaining tryptamine-2-C^{14} was extracted by toluene. The plant diamine oxidase reaction was linear with enzyme concentrations over a range of 0 to 0.20 ml of enzyme preparation (equivalent to 0 to 600 μg of fresh tissue) and substrate concentrations of 5, 50, and 300 mμmole (0.0125 to $0.75 \times 10^{-3}M$).

Results (Table 1) show that BOH is a potent inhibitor of pea-seedling diamine oxidase. In repeated experiments, inhibitory effects of BOH would duplicate very well for an individual experiment; however, the concentration of BOH which would give 25 to 75 percent inhibition BOH without prior treatment of the enzyme preparation ranged from 3.3×10^{-7} to $1.0 \times 10^{-6}M$, depending on the amount of the enzyme preparation used. The concentration of BOH appeared to be reduced somewhat by constituents in the extracts other than diamine oxidase, possibly by free aldehyde groups. Experiments to determine the nature of BOH inhibition of tryptamine oxidation are summarized in Table 2. If the inhibitor and substrate were added to the enzyme preparation at the same time, the degree of inhibition varied in a competitive fashion with enzyme concentration. In contrast, if the enzyme preparation was treated first with inhibitor (at a final BOH concentration of $3.3 \times 10^{-7}M$) the inhibition was noncompetitive. Again, the amount of BOH required for complete inhibition of diamine oxidase activity varied slightly with the amount of enzyme preparation. However, in every experiment, the percentage of inhibition after treatment was always independent of substrate concentration.

The nature of BOH inhibition of tryptamine oxidation is very similar to that for isopropylhydrazine (*7*) with histamine and cadaverine as substrate for diamine oxidase. I found that, by the assay procedure of Okuyama and Kobayashi (*9*) for animal diamine oxidase, the enzyme preparation from pea seedlings would oxidize putrescine at a rate 100 to 200 times greater than that of tryptamine. Similar substrate specificities for these amines were reported by Hill and Mann (*6*) and Werle *et al.* (*7*). In addition, the concentration of BOH necessary to inhibit the oxidation of putrescine to Δ^1-pyrroline was essentially the same as that required for inhibition of tryptamine oxidation.

The inhibition of plant diamine oxidase by BOH allows speculation on the ability of this growth retardant to force flowering of pineapple plants. Gordon and Nieva (*4*) have shown that tryptophan and tryptamine are converted to indoleacetaldehyde by pineapple brei. Thus, in pineapple, diamine oxidase may participate in maintaining indoleacetic acid concentration. Relatively small amounts of BOH, when applied to pineapple plants, may completely inhibit diamine oxidase and cause the auxin concentration to decrease. If auxin synthesis occurs by way of the decarboxylation of tryptophan to tryptamine, one might expect tryptophan decarboxylase, a pyridoxal phosphate–dependent enzyme, to be sensitive to BOH inhibition. Although specific information on the inhibition on this enzyme by hydrazines is not known to me, amino acid decarboxylases in general are not appreciably inhibited at hydrazine concentrations less than $1 \times 10^{-5}M$ (*10*). In studies with animals I have found that when administered intraperitoneally to rats 30 minutes prior to the intraperitoneal administration of putrescine-1,4-C^{14}, BOH (3.8 mg/kg body wt.) can completely inhibit, for more than 6 hours, the conversion of this diamine to respiratory $C^{14}O_2$. In

Table 2. The effects of substrate concentration and prior treatment of enzyme with BOH on tryptamine-2-C^{14} oxidation.

Tryptamine (mμmole)	Indoleacetaldehyde-2-C^{14} formed (mμmole)				
	Control	BOH* added with substrate	Inhibition (%)	BOH* added to enzyme 15 min prior to substrate	Inhibition (%)
25	0.47 ± .001	0.11 ± .01	77	0.01 ± .01	98–100
50	0.81 ± .06	.21 ± .01	74	.01 ± .01	98–100
150	1.73 ± .03	.61 ± .00	65	.03 ± .03	98–100
300	2.48 ± .12	1.09 ± .03	56	.05 ± .05	98–100

* BOH concentration was $3.3 \times 10^{-7}M$. Assay mixture contained tryptamine-2-C^{14} (109,300 DPM) in borate-phosphate buffer 0.1 ml enzyme preparation 0.1 ml and either BOH in buffer, or buffer for a total volume of 0.4 ml. The enzyme was incubated with or without BOH for 15 minutes at 26.5° ± 0.5°C and then substrate was added and the mixtures incubated again for 30 minutes in 26.5° ± 0.5°C. The product formed (mμmole) is expressed as mean ± average deviation of duplicate samples. Data are corrected for blank values obtained by incubating tryptamine-2-C^{14} with boiled enzyme.

control experiments, normal rats metabolized 50 percent of the administered C^{14} to respiratory $C^{14}O_2$ during the same period.

Although several hydrazine derivatives will force flowering in pineapple, many will not (*3*). In our laboratory we have found that methylhydrazine is converted rapidly to methane and CO_2 after administration to intact rats (*11*). These results may suggest that many hydrazine derivatives are not sufficiently stable to remain intact for very long periods after administration to animals and possibly plants.

Of considerable importance is the effect the 1,1-dimethylhydrazide growth retardants—B-nine (B-995) *N*-dimethylamino succinamic acid and *N*-dimethylaminomaleamic acid (C-011)—may have upon plant diamine oxidase. In this regard the parent compound 1,1-dimethylhydrazine is a potent inhibitor of animal diamine oxidase (*12*). Work by Martin *et al.* (*13*) with *N*-dimethylamino succinamic acid-2,3-C^{14} (B-nine) shows that although this growth retardant is quite mobile and stable in apple seedlings it does undergo slow decomposition. Dahlgren and Simmerman (*14*) have reported that C-011 undergoes a slow hydrolysis in aqueous solutions to yield 1,1-dimethylhydrazine and 1,1-dimethylhydrazinium hydrogen maleate. Recent work by Reed, Moore, and Anderson (*15*) shows that inhibition of shoot elongation in dwarf and tall peas by B-995 can be correlated with inhibition of tryptamine-2-C^{14} oxidation to indoleacetaldehyde-2-C^{14} in homogenates prepared from epicotyls of young B-995-treated plants.

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Autosomally Determined Polymorphism of Glucose-6-Phosphate Dehydrogenase in Peromyscus

Abstract. *Glucose-6-phosphate dehydrogenase commonly occurs in at least two forms in most tissues of the deer mouse,* Peromyscus maniculatus, *as demonstrated by starch-gel electrophoresis. An autosomally determined genetic polymorphism in one of the enzymes was discovered. A hybrid molecule occurs in the heterozygote; this suggests a dimeric structure of this enzyme.*

Genetic variants of glucose-6-phosphate dehydrogenase (G-6-PD), demonstrated by altered electrophoretic mobilities in starch gel, have been reported in man (*1*) and *Drosophila* (*2*). In both organisms, the gene controlling this enzyme is located on the X chromosome. It has been suggested (*2, 3*) that X-linkage of the G-6-PD gene may be a general phenomenon and that it may confer some biological advantage which is not now apparent. We report here a G-6-PD polymorphism in the deer mouse, *Peromyscus maniculatus*, which is controlled by an autosomal gene.

The animals were obtained from a laboratory population. Some, descended exclusively from mice trapped in Washtenaw County, Michigan, by Van T. Harris in 1946 and 1947, belong to the subspecies *P. m. bairdi*. Others are hybrids between members of this stock and other subspecies of *P. maniculatus*. Animals from four distantly related sibships were examined, together with their parents. All animals were at least 2 months old, and females were not pregnant or lactating.

The mice were killed by decapitation; the organs were removed immediately, washed in cold physiological saline solution, blotted, weighed, and homogenized in a Kontes glass tissue-grinder with a measured amount of distilled water. Cell walls were ruptured by high-frequency sound. Solid material was removed by centrifugation at 25,000*g* for 10 minutes at 4°C. The clear supernatant was pipetted into the starch-gel insert slots in 25-μl amounts.

At least two different zones of G-6-PD activity were found in all tissues examined except erythrocytes. These were arbitrarily designated as A and B enzymes, the A form being the more anodally migrating (Fig. 1). Both occurred in liver, kidney, testis, muscle, lung, heart, and brain. In erythrocytes, only the A form was present.

In three of the four families studied, a polymorphism occurred which involved the B enzyme. Three phenotypes were observed: in two, B-a and B-b, there was a single band at one of two different positions; in the third, phenotype B-ab, bands appeared at both of these two positions with a third halfway between (Fig. 1). In this type, the middle band was relatively more intense than the other two.

Table 1 shows the results of the four matings. Although the hypothesis of partial sex-linkage of the gene controlling the B enzyme of G-6-PD cannot be excluded, complete sex-linkage involving a single locus is ruled out because in one sibship, from a mating between B-ab animals, males of all three phenotypes appeared. Additional

Table 1. Glucose-6-phosphate dehydrogenase patterns of parents and offspring in four matings.

G-6-PD phenotypes of parents		G-6-PD phenotypes of offspring							
		B-a		B-ab		B-b		Total	
♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
B-b	B-b					4	2	4	2
B-a	B-ab	1	3	1	1			2	4
B-ab	B-b				4	2	1	2	5
B-ab	B-ab	1	2	2	5		2	3	9