hour in the absence of molar $MgCl_2$. This protective effect was shown to be due to the presence of suckling mouse tissue extract and was not an inherent property of these types. Coxsackievirus types A2 and A4 and echovirus type 9 as infected suckling mouse tissue extracts and as infected cell culture fluids were tested for $MgCl_2$ stabilization. It was found that in contrast to suckling mouse tissue materials, the viruses in cell culture fluids were readily inactivated by the heat treatment in the absence of molar $MgCl_2$, but stabilized in the presence of the salt. Furthermore, MK-grown coxsackievirus type A9 could be protected against thermal inactivation by suspending it in normal newborn mouse tissue extract.

Summary. The susceptibility of primary cultures from monkey kidney, human embryonic kidney, human embryonic lung, and human amnion cells, and of WI-38 and KB cells, to 23 prototype strains of group A coxsackieviruses was studied. All types except A1, 5, 6, 19 and 22 could be propagated in one or more of the various cell cultures used. The property of $MgCl_2$ stabilization was established for all 18 prototype viruses that can be grown in tissue culture.

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Mutation in *Drosophila* by Methylazoxymethanol, the Aglycone of Cycasin.* (32256)

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Cycasin, methylazoxymethanol-beta-glucoside, is a naturally occurring compound that has been isolated from cycad plants(1). Cycasin has been found to be toxic and carcinogenic when fed to conventional laboratory rats but not when fed to germfree rats(2). The evidence indicates that in germfree rats beta-glucosidase is not present to hydrolyze cycasin and release the aglycone, methylazoxymethanol (MAM), which is toxic and carcinogenic in rats(3,4), induces reverse mutation in *Salmonella*(5), and is an active alkylating agent(6). Cycasin is radiomimetic in *Allium* (onion) seedlings(7) which show beta-glucosidase activity. The present study reports the results of tests for mutagenesis in *Drosophila* using the induction of sex-linked recessive lethals in males from feeding cycasin, MAM, and MAM-acetate.

Material and methods. Tests for sex-linked recessive lethals in *Drosophila melanogaster* were carried out using the Muller-5 technique. Wild type males which were subjected to chemical treatment were taken from the Canton-S strain. *Drosophila* were reared on the corn meal, molasses, dried yeast, agar, propionate medium of Sandler. The medium was inoculated with the live propionate-resistant Y-2 yeast strain of Wagner.

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For chemical treatment wild type males from the Canton-S strain were starved for 24 hours and allowed to feed on experimental solutions for one hour. Average uptake of chemical solution was determined by weighing groups of 12 to 24 males before and after they had fed. Dose of chemical was varied by using different concentrations of test substances. MAM was prepared and fed as the incubated solution of cycasin plus beta-glucosidase (almond emulsin). Control males for cycasin treatment were fed glucose; MAM controls were fed glucose plus emulsin; and MAM-acetate controls were fed glucose in order to make them comparable to other controls. Control flies fed glucose or glucose plus emulsin took up amounts of these materials that corresponded closely to the uptake by experimental flies. Following ingestion of chemicals, males were mated during 2 to 4 days to exhaust mature sperm and then mated with Muller-5 virgin females. Second generation progenies were scored for sex-linked recessive lethals. Progenies in which 9 or more bar-apricot phenotype and no wild type males emerged were scored as "lethals" since the probability is very high that each such progeny resulted from a treated chromosome in which a sex-linked lethal had been induced. Progenies of similar size were scored as normal or "no lethal" if one or more wild type males was found.

Samples of incubation mixtures of cycasin and emulsin were spotted on silica gel thin layer plates at the beginning and end of the feeding period in order to check both for completion of cycasin hydrolysis to MAM and to verify that the labile MAM had not been destroyed. The plates were irrigated using butanol:acetic acid:water (4:1:1) and azoxy-compound spots were developed by spraying with a chromotropic acid sulfuric acid solution(8) and heated at 105-110°C for 10-15 minutes. MAM, MAM-acetate, and cycasin made purple spots.

In as much as mutagenesis, carcinogensis, toxicity, and radiomimetic effects variously appear to be associated with free aglycone activity, the enzymatic potential of *Drosophila* and the larval food yeast for releasing MAM from cycasin and MAM-acetate was assessed. Homogenized flies and yeast were tested for beta-glucosidase using p-nitrophenyl-beta-glucoside, and for esterase using p-nitrophenylacetate. Both substrates were incubated with homogenates for 30 minutes in 0.1 M triethanolamine buffer at pH 7.0. The reaction was stopped by adding ethanol and the color of released p-nitrophenol was determined at pH 8.5 using absorbance at 395 mμ. Beta-glucosidase and esterase tests were also carried out on *Drosophila* and yeast homogenates utilizing MAM acetate and cycasin as substrates and using thin layer chromatography to check for MAM formation.

Results. There experiments were performed. Cycasin and MAM-acetate were both tested in two experiments and MAM in a third. The incidence of sex-linked recessive lethals is shown in Table I. Cycasin was not significantly mutagenic in either experiment, nor was MAM-acetate in experiment a; however, MAM and MAM-acetate were highly significantly mutagenic (P = less than 0.005) by the Chi Square test in later experiments.

Feeding on experimental solutions resulted in reduced viability of wild type males. Virtually all the males that died following ingestion of chemicals did so within 4 days. The ratios of dead males to total males at 4 days after feeding were: control, 1 dead of 76; cycasin, 9 dead of 96; MAM-acetate, 34 dead of 50; and MAM, 34 dead of 70. It is likely that there were individual differences in uptake of chemicals by this feeding technique. The dead flies might have been ones that had drunk more of the solution, and the surviving flies thus have been ones that had consumed lesser amounts. The pooled mutation frequencies can therefore be regarded only as general indices of mutagenesis.

The pretest matings to exhaust mature sperm, *i.e.,* postmeiotic stages, may have reduced the observed frequency of sex-linked recessive lethal mutations in as much as postmeiotic stages of *Drosophila* sperm have been found to be more sensitive to an alkylating mutagen than earlier stages(9).

Tests of homogenates of *Drosophila* and yeast indicated that substantial esterase activity was present in both and that beta-

TABLE I. Incidence of Sex-Linked Lethal Mutations in *Drosophila* from Feeding Cycasin, Methylazoxymethanol, and Methylazoxymethanol Acetate.

Treatment	Experiment	Avg uptake of chemical per fly (μg)	No. mutants	Total No. chromosomes tested	Avg mutants (per 1000 chromosomes)
Cycasin	a	7.7	3	232	
	a	.76	1	239	
	b	9.3	1	883	
			5	1354	3.7
MAM-acetate	a	.11	1	138	
	b	.054	9	352	
	b	.047	8	343	
			18	833	21.6
MAM	c	.17	5	101	
	c	.09	7	220	
	c	.10	13	244	
			25	565	44.3
Control	a	—	0	236	
	b	—	0	326	
	c	—	4	564	
			4	1126	3.6

glucosidase was very low or absent. Thus, consumption by the flies of MAM-acetate would be expected to give rise to MAM, but cycasin should not have formed MAM unless the gut flora developed beta-glucosidase-containing strains of yeast or other organisms. The flies tested for beta-glucosidase had not been fed cycasin. It is known that although cycasin is not toxic to germfree rats, it is toxic to exgermfree rats whose guts have been infected with a single species that contains beta-glucosidase(3). Cycasin, although not mutagenic in these experiments, was associated with a 10% mortality of fed males compared to 1.5% for the controls. The lethal effect of cycasin feeding on male flies could derive from the occasional development of a beta-glucosidase-containing gut organism over a period of 2 to 4 days, which, when it occurred, was lethal to the fly. A mutagenic effect of cycasin feeding in *Drosophila* might be dependent upon mouthpart or gut flora.

Summary. A naturally occurring compound, cycasin (methylazoxymethanol-beta-D-glucoside), and its aglycone and the aglycone acetate were tested for the induction of sex-linked recessive lethal mutations in *Drosophila*. Cycasin was not mutagenic but methylazoxymethanol and its acetate were potent mutagens.

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