well as from the work of Buchanan (*4*) and of Hall and Long (*5*), the reduced organic materials (those extractable by acetone and benzene) average 10 ± 5 percent. From the value of 10 percent "oil" content, the following rough calculation can be made: For biomass production an annual yield per acre of 10 tons (dry) can reasonably be expected (*2*), giving an oil production of 1 ton (7 barrels). The annual cultivation costs per acre are estimated to be $150 (*2*), or about $20 per barrel. Processing costs are not included, but neither is the value of the residual biomass. The possible added value of the oil for uses other than fuel has also yet to be determined. This preliminary cost estimate is close enough to the current price of petroleum to warrant further investigation. Test plantings in California of *Euphorbia lathyris* (of which we had sufficient seeds) and of *Euphorbia tirucalli* (whose cuttings were readily available to us) have been started, and from these we can try to confirm and refine the above figures. An experimental planting of *E. lathyris* at the South Coast Field Station of the University of California in Santa Ana has so far indicated a yield of not less than 8 barrels per acre in the 7-month growing season from February to September.

Table 2 shows that the latex of these plants is very rich in reduced photosynthetic materials (polyisoprenes and sterols), which comprise up to 80 percent of the dry weight (the latex, depending on the species and on the time of year and day, contains 30 to 90 percent water). Furthermore, some of the species are trees (such as *Achras sapote, Euphorbia tirucalli,* and *Euphorbia trigona*), and they could therefore be tapped. As compared to *H. brasiliensis*, which has to be hand-tapped, an automatic, continuous flow device (reducing the costs considerably) might be constructed for these species. Their latex contains much less rubber and more lower molecular weight material than *H. brasiliensis*, and therefore coagulates much less readily.

Although the results of this research are still fragmentary, the possibility of future "petrochemical plantations" is becoming apparent.

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12 April 1977; revised 15 August 1977

Saccharin and Other Sweeteners: Mutagenic Properties

Abstract. *Saccharin preparations commonly distributed as artificial sweeteners exhibited mutagenic activity in bacterial tests. When administered orally to mice, mutagenic activity was demonstrable in the urines of these animals as well as in a host-mediated assay. Highly purified saccharin was not mutagenic in the direct assay, but the urines of mice to which this material had been administered exhibited mutagenic effects on one tester strain (*Salmonella typhimurium *TA100). Two other sweeteners, neohesperidin dihydrochalcone and xylitol, had no detectable mutagenic activity in any of these assays using his*$^-$ Salmonella typhimurium *strains TA100 or TA98.*

Although reports of 17 studies are available, conclusive evidence is lacking as to whether saccharin is mutagenic (*1*). This problem was reinvestigated by more sensitive methodologies (*2*) and tester strains—his$^-$ *Salmonella typhimurium* TA100 and TA98—developed by Ames *et al.* (*3*) for the detection of base-pair substitutions and frameshift mutations, respectively. Several saccharin preparations, as well as two other sweeteners, neohesperidin dihydrochalcone (NHDC) (*4*) and xylitol, were examined.

The following six saccharin preparations were tested: (i) a preparation, obtained from a local pharmacy, which is representative of the material distributed to the consumer as a pharmaceutical preparation (A); (ii) a sample manufactured by Sherwin-Williams Company and supplied by a wholesale pharmaceutical distributor (B); (iii) a sample, provided by Dr. D. Stoltz, from a lot (S-1022) that was used in a recent carcinogenic study (*5*) (C); (iv) a highly purified sample of C, also donated by Stoltz (D); (v) a powder containing 40 mg of saccharin and 1 g of lactose–potassium tartrate filler enclosed in a widely used and freely available convenience package (Sweet 'N Low) (E); and (vi) a sample, provided by Stoltz, from another lot (S-1233) used in the above-mentioned carcinogenic study (*5*) (F). Xylitol was supplied by Sigma, and NHDC was a gift from Dr. R. Horowitz.

Mutagenic activities were determined directly, as well as in the host-mediated assay, and in the urines after oral administration of the test substances, as in a previous study concerned with antischistosomal drugs (*6*). The direct assays of the compounds were conducted at several concentrations, both in the absence and presence of a rat liver microsomal fraction (S_9) (*7*). In the host-mediated assay, the bacterial tester strains were incubated for 6 hours in the peritoneal cavity of mice to which the test substance had been administered by gastric intubation (host-mediated assay) (*2*). The second type of assay in vivo consisted in the analysis of urines that had been collected for a 24-hour period after the oral administration to mice of the test compound. The mutagenic activities of 0.1- and 0.25-ml samples were assayed for each urine without prior concentration. All determinations were conducted in duplicate, and all observed positive mutagenic effects were dose-related.

After the oral administration of saccharin preparations A, B, and C, significant mutagenic activities in the urines were detectable with both *Salmonella* strains TA100 and TA98 (Table 1). Activities for TA98 were markedly enhanced after incubation of the urines with β-glucuronidase. In the case of TA100, unconjugated and conjugated mutagens were inactivated by liver microsome (S_9) preparations. By contrast, the mutagenic effects of the urines on TA98 were greater in the presence of S_9 (Table 1). Therefore, it would appear that two different mutagenic substances were present.

No mutagenic activity was found with TA98 in the urines of mice treated with the highly purified saccharin preparation D. However, there was low, but significant, mutagenic activity with the tester strain TA100. In addition, these urines contained a conjugated promutagen,

which was activated by S_9 after β-glucuronidase treatment. Apparently the presence of this substance in the less purified preparations was masked by the greater mutagenic activity in these urines. Histidine in the urines could not account for the increased number of revertants, because, after the administration of saccharin, the concentration of this amino acid was unchanged (approximately 40 μg/ml), as determined both by amino acid analysis and by microbiologic assays with *S. typhimurium* strain TA850 (*8*). Furthermore, since counted colonies grew on histidine-deficient plates, they proved to be true revertants. The low mutagenic activity of the purified preparation D may be due either to a residual contaminant or to a metabolite of saccharin per se. The determined urinary mutagenic activities represent minimal values because incubation of saccharin in urine for 24 hours at 4°C resulted in a 10 to 15 percent loss of mutagenic activity with TA100 and a somewhat lesser reduction for TA98 (*9*). This should be taken into consideration, because the mouse urines were collected over a 24-hour period in a vessel surrounded with ice.

In contrast to saccharin preparations, urines of animals treated with the other two sweeteners, xylitol and NHDC, exhibited no mutagenic activity with TA100 or TA98 under either of the two test conditions (Table 1). Conversely, saccharin preparations A, B, and C also induced significant host-mediated mutagenic activity; this was not the case with purified saccharin (D).

In order to avoid interference by growth inhibition, the maximal saccharin concentration at which mutagenic activity of each sample assayed in vitro was approximately 20 percent lower than the concentration producing 10 percent growth inhibition. The less purified saccharin preparations were more toxic to the bacteria than the highly purified saccharin; also, addition of S_9 reduced growth inhibitory effects of the less pure preparations.

There was considerable variation in the mutagenic activities in vitro of the five saccharin preparations (Table 2). However, only the highly purified preparation (sample D) failed to exhibit any significant mutagenic activity. Two preparations (A and B) were activated by S_9, while there was no activation with two other preparations (C and E). Another preparation (F) was activated by S_9 when tested with one strain (TA100) but not with another (TA98).

These qualitative and quantitative differences in the mutagenic effects among a variety of saccharin preparations and the absence of mutagenic activity in vitro in the purified sample confirm the report of Stoltz *et al.* (*10*) that commercial saccharin samples contain mutagenic impurities. Significant mutagenic activity was detected by both tester strains with as little as 5 mg per plate or one-eighth of a single packet of preparation E, while the packet content in its entirety usually is added to a single beverage-containing cup. By contrast, 24 to 100 times higher amounts per plate of NHDC or xylitol, respectively, were completely negative. Thus, with these two compounds no mutagenic activities were detectable, at concentrations far exceeding levels at which there were mutagenic effects with those saccharin preparations (A, B, and E) that are consumed by the public.

The weak, albeit significant, mutagenic activities in vitro and in vivo of preparation C correlate with the weak carcinogenic activity of the same preparation reported recently (*5*). In contrast, NHDC has been found to be devoid of both

Table 1. In vivo mutagenic activities (MA) of orally administered (2.5 g/kg) saccharin preparations and two other sweeteners. The assay by Ames *et al.* (*7*) was used, except that biotin and L-histidine were placed in the bottom (15 ml), rather than the top (2 ml) agar phase and that the amounts of glucose (22 and 176 mg for TA100 and TA98 per plate, respectively) and of L-histidine (100 μg per plate) were modified in order to maximize sensitivity. Furthermore, in the top agar 0.9 percent NaCl was replaced by Vogel-Bonner medium (*12*). In this phase, the agar concentration was 0.75 percent. Bacterial suspensions were grown in double-strength nutrient broth (Difco) at 37°C for 14 hours with agitation in a Dubnoff metabolic shaker.

Compound and preparation	Revertants induced by urines excreted per mouse over 24 hours after treatment*				Intraperitoneal host-mediated MA revertants†	
	TA100		TA98			
	−β-Glucuronidase	+β-Glucuronidase	−β-Glucuronidase	+β-Glucuronidase	TA100	TA98
Saccharin A	1840	1860	330‡	1330‡	207	69
Saccharin B§	900	1430	240‡	820‡	177	25
Saccharin C	1250	1370	100‡	260†	53	7‖
Saccharin D	350	600‡	0	0	11‖	0
Xylitol	0	0	0	0	0	0
NHDC	0	0	0	0	0	0

*If addition of a rat liver microsome fraction (S_9) resulted in an increase in the number of revertants, the latter figure is recorded. †Per 10^8 bacteria, in excess of controls; each figure represents the average of five mice. Spontaneous revertants amounted to 58 ± 10 and 22 ± 2 for TA100 and TA98, respectively. ‡S_9 (Aroclor induced) added. §The MA of this preparation did not differ significantly from those of a sample obtained from Sigma (St. Louis, Mo.). ‖Not significant.

Table 2. In vitro mutagenic activities of saccharin preparations, NHDC, and xylitol; N.S., not significant.

Substance	Amount (milligrams per plate)	Number of revertants in excess of controls			
		TA100		TA98	
		$-S_9$	$+S_9$	$-S_9$	$+S_9$
Saccharin A	2	17	39	N.S.	36
	5	51	81	11	92
	15		235		277
Saccharin B*	2	22		N.S.	
	5	54	29	10	26
	10	96		22	
	15	147	100	29	62
Saccharin C	2	13		N.S.	N.S.
	5	31	12	11	10
	15		36		30
Saccharin D	32	N.S.	N.S.	0	N.S.
	80	0	10	0	N.S.
Saccharin E†	2	22		17	
	5	50	32	38	22
	15		67		44
Saccharin F	10	10	47	18	N.S.
	20	45	103	38	12
	30		155		17
NHDC	40	0	0	0	0
	120	0	0	0	0
Xylitol	200	0	0	0	0
	500	0	0	0	0

*See footnote (§) in Table 1. †The mutagenic activity of this preparation was similar to that of the content of another brand of convenience package (Twin Sugar).

mutagenic and carcinogenic (*11*) activity. These data provide additional support for the predictive value of mutagenic assays.

Additional toxicological tests conducted with NHDC and xylitol would provide evidence whether or not safer alternatives to saccharin as sweeteners are already available.

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1 July 1977

Macronuclear Subunits of *Tetrahymena thermophila* Are Functionally Haploid

Abstract. *In* Tetrahymena thermophila *the major argument for the existence of diploid subunits has been that some loci show a delay in the accumulation of stable subclones during macronuclear assortment. This delay is based on the assumption that throughout the life cycle there are 45 subunits. We find that for at least 50 fissions after conjugation there is sufficient DNA for 66 haploid subunits. These additional subunits early in the life cycle are sufficient to explain the observed accumulation of stable subclones in all instances. This removes the need to invoke diploidy to explain assortment, thus resolving the question of subunit ploidy in favor of haploidy.*

Although there is consensus that the macronucleus of *Tetrahymena thermophila* (*1*) consists of independent subunits, the genetic organization of these subunits has been unclear. Equally persuasive arguments for their organization as either haploid or diploid sets of micronuclear genes, as well as arguments that they are individual gene loci, have been presented (*2*). Resolution of this problem is of special importance not only because of the current interest in the evolution of the ciliate macronucleus (*2*), but also because *Tetrahymena* is increasingly used in genetic studies (*3*). In this report we present evidence that the argument for diploidy is based on an illusion created by the presence of 66 rather than 45 subunits early in the life cycle. We conclude that macronuclear subunits behave as haploid sets of genes throughout the life cycle.

The existence of subunits is inferred largely from the phenomenon of macronuclear assortment (*2-6*). With the exception of one locus (*7*), all heterozygotes, regardless of dominance relationships, are initially unstable for phenotype (which in ciliates is controlled by the macronucleus) and give rise to stable subclones which irreversibly express only one allele. The available evidence is best explained by a stochastic model (*4, 8*) in which, following their replication, subunits are randomly distributed at each macronuclear division. Thus, in an unstable heterozygote there are two kinds of subunits, those expressing one gene and those expressing its allele. With repeated random subunit distribution stable subclones whose macronuclei consist of only one type of subunit are eventually produced (*9*). This model allows calculation of the number of assorting units from the equilibrium rate of assortment (*10*). For all assorting loci, the equilibrium rate of assortment is experimentally the same (*3*) and implies the presence of 45 subunits in a G_1 macronucleus. The observation that assortment for all loci yields 45 subunits suggests a single common mechanism.

Since macronuclear DNA behaves with haploid reassociation kinetics (*11*), and since G_1 macronuclei have an average DNA content of 45C (*12, 13*), the conclusion that there are indeed 45 haploid subunits would appear straightforward. However, macronuclear assortment also provides a powerful argument in favor of diploidy (*2, 3, 6, 13, 14*). Specifically, for some loci the accumulation of stable subclones is significantly slower than expected if 45 subunits were to begin the assortment process immediately after conjugation. It has been argued (*5, 15*) that, since for these late-assorting loci the products of both alleles can be detected in all clones for 30 to 50 fissions after conjugation, the delay must mean that subunits are heterozygous and, therefore, diploid. It was proposed that either a genetic mechanism to delete an allele or an epigenetic mechanism to repress permanently one allele had to exist (*5*).

Several attempts to reconcile the diploidy implied by late assortment with the concept of haploid subunits have been made, but none has been successful (*13, 16-18*). The hypothesis that 23 diploid subunits might in some way mimic the assortment of 45 haploid subunits is inconsistent with the random nature of assortment (*8, 19*).

The resolution proposed here is based on the results of cytofluorimetric measurement of the DNA content of G_2 macronuclei of heterozygous cells (*20, 21*). For the first 50 fissions after conjugation the mean DNA content of G_2 macronuclei is 132C (Fig. 1). This is considerably more than the 90C found in older cells and is sufficient DNA for 66 haploid subunits in a G_1 macronucleus. The importance of these subunits is made clear in Figs. 2 and 3. Figure 2 shows that when the ratio of types of subunits is close to 1 : 1 at the initiation of assortment, the accumulation of stable subclones (also in a 1 : 1 ratio) is initially slower than for other ratios. For ratios that are close to 1 : 1, as are the ratios observed for stable types for all late-assorting loci (*3*), the accumulation of stable types is a direct function of the number of subunits (Fig. 3). For 66 subunits there is an additional delay of 10 to 15 fissions in the accumulation of stable subclones as compared to the assortment of 45 subunits.

It is our contention that these additional subunits early in the life cycle are sufficient to account for the reported delay in assortment. Figure 3 shows the reported proportions of stable subclones for all late-assorting loci for which naturally occurring codominant alleles are available (*22*). Despite the fact these proportions represent small numbers of stable subclones, the agreement with the assort-