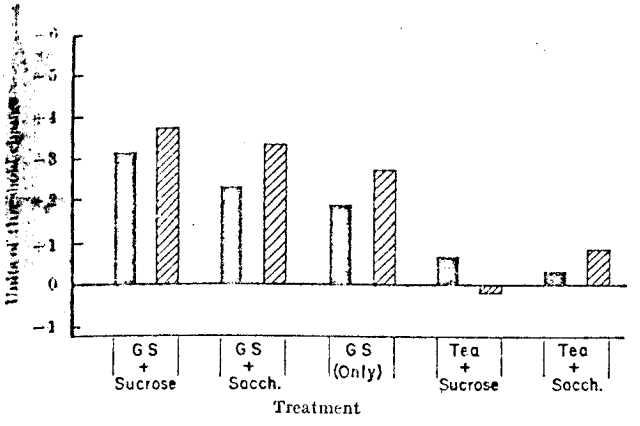

Fig. 1. Effect of *Gymnema sylvestre* and sweet adaptation on sucrose (black columns) and sodium saccharin (hatched columns) thresholds.

0·5 M sucrose or 10^{-3} M sodium saccharin; GS extract with either sucrose or sodium saccharin present in adapting concentrations; three rinses with adapting solution; three rinses with 50 ml. water. Six minutes after the GS extract in sucrose or saccharin was spat out, there was a final water rinse. Thresholds were immediately redetermined for both saccharin and sucrose.

A 2 per cent instant tea preparation similar in appearance, taste and aroma was substituted for GS extract as a control. The treatment and rinsings were otherwise the same as already described. A series of sixteen solutions increasing in concentration by $\sqrt{2}$ were available for sucrose ($2{\cdot}2 \times 10^{-3}$ M to 0·4 M) and also for saccharin ($4{\cdot}3 \times 10^{-6}$ M to 8×10^{-4} M). The GS extract was prepared by a modification of a process described elsewhere[6]. Six subjects were selected for their ability to distinguish the tastes of NaCl, HCl, sucrose and caffeine, recognize changes in supraliminal concentrations of these solutions, and the reliability of their threshold determinations. Changes in threshold (Fig. 1) are expressed in terms of the solution steps used in this study: each unit represents a $\sqrt{2}$ change.

Adaptation to either strong solutions of sucrose (0·5 M) or sodium saccharin (10^{-3} M) did not affect the elevation of threshold produced by GS extract without the sweet additives. Indeed, values were higher when adaptation to sweet accompanied GS treatment but this apparent "potentiation" did not reach significance in any case (two-tailed *t*-tests).

When the tea placebo was used with sucrose and with sodium saccharin adapting solutions, little residual adaptation was observed, indicating that the procedure of multiple water rinses eliminated almost all influence of prior adaptation, but not the effect of treatment with GS extract.

It should be noted that the greater effect of GS extract on elevation of sodium saccharin thresholds as compared with sucrose thresholds is in agreement with the fact that the sweetness of sodium saccharin increases more slowly than sucrose with increasing concentration. Hence, the threshold concentrations of these two substances following GS extract would correspond to approximately equisweet supraliminal solutions before treatment[6].

Taste receptors may respond to a stimulus within 30–50 ms. It has therefore been suggested that sapid substances do not readily enter the interior of the cell, but rather react at the cell surface, on particular sites in the microvilli which may be capable of binding molecules and ions[8]. The results indicate that suppression of sweet sensitivity by GS extract is probably not a matter of competition for receptor sites, but it does not exclude the possibility of a mechanism involving a non-competitive site which may alter a configuration necessary for stimulation. The persistence of the effects of GS extract, however, even after repeated rinsing, long after the effects of sucrose and saccharin adaptation would have worn off, suggests that it may interfere with one or more of the intermediate steps in the essential cellular sequence leading to nerve stimulation.

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Lethal Mutation Rate in *Drosophila* exposed to LSD-25 by Injection and Ingestion

Although attempts have been made to assess the effect of D-lysergic acid diethylamide (LSD-25) on human chromosomes, it has yet to be unequivocally determined when and in what circumstances this chemical is mutagenic. From karyotype analyses of individuals who have ingested LSD-25 some investigators have concluded that it is not mutagenic[1,2] while others have reported a high incidence of chromosome aberrations among such individuals[3,4] as well as among human leucocytes treated *in vivo*[5]. Because of its long standing utility in the detection of induced mutations *Drosophila* seems to be a likely organism with which to investigate this problem further. Reports have recently appeared which indicate that while LSD-25 is not apparently mutagenic when injected directly in *D. melanogaster* males in amounts as great as 500 μg/ml. (ref. 5) it is capable of producing X-linked recessive lethal mutations when doses some twenty times larger are used[6].

One may ask, however, if the effects of this chemical are dose-dependent, if it is equally effective on both the *X* and autosomes and if it is mutagenic when introduced to the adult by ingestion as well as injection. Injection is the most direct means of introducing this chemical to the gonads and ingestion (the usual human method of exposure) permits the administration of relatively large repetitive doses. Two procedures were used for the detection of lethal mutations, the Muller-5 (*M*-5) technique[7] which detects *X*-linked recessive lethals, and the Curly-Lobe/Plum (*CyL*/*Pm*) technique which detects second chromosome recessive lethals[8]. In all tests the flies were taken from a highly inbred line. Males 36–48 h old were injected with LSD-25 at either of two concentrations, 100 and 2,000 μg/ml. The lesser concentration was obtained already dissolved in 1 ml. aqueous stock solution (Sandoz Batch No. 65002); the higher concentration was prepared from LSD-25 powder (Sandoz Batch No. 88601) diluted with the stock solution. Each male received an abdominal injection of about 0·2 ml. of solution. (The control males received injections of the stock solution.) At the concentrations used the amounts of chemical injected correspond, respectively, to about 24 and 470 μg per g of body weight.

Twenty-four hour old males were placed in empty vials for 12 h before treatment to ensure some degree of de-

hydration. Each fly was then immobilized with ether. A graduated micropipette was held to the proboscis and as the fly recovered it drank some of the solution. The amounts of liquid ingested ranged from 0·7 to 1·5 μl. ($\bar{x}$ = 1·2 μl.). The treated flies were then allowed to feed on a *Drosophila* culture medium for about 2 h, after which time they were twice more dehydrated and fed the test solution. Depending on the concentration of the liquid (100 or 2,000 μg/ml.) a fly received approximately 420 or 8,460 μg LSD-25 per g of body weight (or some eighteen times more than that introduced by injection). The control males were fed the stock solution.

After exposure by either method a male was mated to a virgin female of the appropriate genotype every 3 days to produce a total of three broods. This procedure permits detection of postmeiotic (days 0–7) and to a lesser extent meiotic (days 8–9) mutations. For each treatment twenty fertile males were tested; about thirty paternal *X* or second chromosomes were isolated from each of the three broods arising from a male for a total of about ninety chromosomes from each.

Table 1. FREQUENCY OF LSD-25 INDUCED LETHAL MUTATIONS

Exposure	Type	Days after treatment 1–3	4–6	7–9	No. of chromosomes	Mean lethal frequency (per cent)
		(Control)				
Injection	*M*-5:					
	Lethal	3	2	2	7	0·39
	Nonlethal	597	591	593	1,781	
		(100 μg/ml.)				
	Lethal	2	0	3	5	0·28
	Nonlethal	590	579	588	1,757	
		(2,000 μg/ml.)				
	Lethal	6	5	3	14	0·79
	Nonlethal	581	590	594	1,765	
		(Control)				
	CyL/Pm:					
	Lethal	0	4	5	9	0·51
	Nonlethal	592	588	573	1,753	
		(100 μg/ml.)				
	Lethal	1	6	3	10	0·57
	Nonlethal	597	564	591	1,752	
		(2,000 μg/ml.)				
	Lethal	8	6	2	16	0·90
	Nonlethal	590	589	583	1,762	
		(Control)				
Ingestion	*M*-5:					
	Lethal	0	1	4	5	0·28
	Nonlethal	577	591	593	1,761	
		(100 μg/ml.)				
	Lethal	5	0	2	7	0·39
	Nonlethal	595	587	598	1,780	
		(2,000 μg/ml.)				
	Lethal	3	7	10	20	1·13
	Nonlethal	572	588	587	1,747	
		(Control)				
	CyL/Pm:					
	Lethal	4	0	3	7	0·40
	Nonlethal	591	586	568	1,745	
		(100 μg/ml.)				
	Lethal	0	4	5	9	0·51
	Nonlethal	592	569	589	1,750	
		(2,000 μg/ml.)				
	Lethal	7	12	14	33	1·89
	Nonlethal	582	586	553	1,721	

Table 1 shows the frequencies of lethals detected within the four chief treatment groups; in each group the frequency of lethals obtained after exposure to the stock (control) solution can be used as the basis of comparison. There is a general increase in the average frequency of lethals with dose within all the chief groups, although in no group is the difference between the mean of the control and the lowest LSD-25 exposure statistically significant.

As Table 2 shows, however, there are some significant differences when one compares the controls to the flies receiving the maximum exposure within each group of treatments. With the exception of the second chromosome lethals scored after injection, there is a significant increase in lethal frequency with maximum dose.

Table 2. SIGNIFICANCE OF DIFFERENCES BETWEEN FREQUENCY OF LETHAL MUTATIONS INDUCED BY INGESTING AND INJECTING LSD-25

Type	Treatment	Mean frequency (per cent) Control	Max. dose	*t*-value	Probability
M-5	Injection	0·39	0·79	2·500	$0·025 < P < 0·05$
	Ingestion	0·28	1·13	2·237	$0·025 < P < 0·05$
CyL/Pm	Injection	0·51	0·90	1·001	$0·20 < P < 0·25$
	Ingestion	0·40	1·89	3·548	$0·01 < P < 0·025$

Within both the injection and ingestion series, the average frequencies of second chromosome lethals scored after a particular treatment exceed that of the corresponding *X*-linked type (Table 1). Because the second chromosome of *D. melanogaster* is about twice as long as the *X*-chromosome, these results suggest that there is no marked difference in the sensitivities of these two linkage groups to the induction of lethals by this chemical. In addition, the frequencies of both *X* and autosomal mutations are, on the average, greater within the ingestion than the injection series; this difference, however, is not statistically significant.

My data suggest, but by no means prove, that there is a threshold dose-response for the mutagenic action of LSD-25 in *Drosophila*; the lowest concentration (100 μg/ml.) produced no significant increase in the frequency of recessive lethals whereas the higher concentration (2,000 μg/ml.) did. Supporting this, Grace *et al.*[3] did not observe a mutagenic effect when LSD-25 was injected at a concentration of 500 μg/ml., while Browning[6], who used a concentration of 10,000 μg/ml., obtained positive results. In addition, G. Markowitz and G. Brosseau (personal communication) performed a statistical analysis of all available data (the data of the present experiment included). While they concluded that a linear dose-response for this chemical could not be completely disallowed, the overall picture that emerges is consistent with the existence of a threshold dose-response effect.

Browning's data[6] suggest that LSD-25 when injected operates chiefly on the mature, that is, postmeiotic, sperm of *D. melanogaster*. Table 1 shows that the males receiving the maximum injected dose produce from two to three times as many lethals in the first as in the last broods, although the opposite trend can be observed in the ingestion series. All broods would have had to be continued a minimum of 11 days to detect premeiotic mutations. Because, however, the first two broods, and to a lesser extent the third, test postmeiotic cells, and because at least one-half of all induced lethals were detected in these first broods, we also conclude that this chemical has a pronounced effect on postmeiotic germ cells in both exposure regimes.

While it appears that lethals can be induced by either injection or ingestion, the relative efficiencies of induction by the two methods differ. Although the ingestion groups received eighteen times more LSD-25 than the injection groups the numbers of lethals produced by either method were not significantly different. It should also be noted that injected males are immediately mated while those fed the chemical are stored for at least 24 h after their first exposure in order to complete the serial feedings. I conclude that, overall, ingestion is not as efficient a mode of exposure as injection. Possibly the LSD-25 is degraded more readily while passing through the gut of the fly than when injected directly into the abdomen, or it may be that the chemical may not readily diffuse to the gonads when ingested.

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