Fig. 1. Biohazard warning symbol. The design color stipulated in the standard form is fluorescent orange-red.

with the psychology of recognition and retention. These criteria, in order of their importance, are that the symbol be (i) striking in form in order to draw immediate attention; (ii) unique and unambiguous, in order not to be confused with symbols used for other purposes; (iii) quickly recognizable and easily recalled; (iv) easily stenciled; (v) symmetrical, in order to appear identical from all angles of approach; and (vi) acceptable to groups of varying ethnic backgrounds. Dow artists created more than 40 symbol designs, of which six were selected for testing. A survey to ascertain acceptability of the six symbols was conducted among Dow employees. This survey was directed toward determining uniqueness and memorability.

To select the final symbol, a nationwide survey, based on precepts well established in mass-psychology experimentation, was conducted in two parts. First, the candidate symbols were tested for uniqueness by determining which had the least prior association for the viewer. Three hundred subjects, males and females, from 25 cities and with various amounts of income and formal education were shown the six symbols along with 18 other commonly used symbols. They were asked what each symbol meant, or was used for. Participants were also encouraged, if uncertain, to guess at the meaning. A "meaningfulness score" was obtained for each symbol based on the percentage of respondents who offered any association whatever to the symbol. Since the purpose was to determine the least meaningful symbol, the smaller scores identified the most desirable symbols.

One week after the initial survey had been conducted, participants were revisited for a "memorability" test. The original respondents were shown a group of 60 symbols which included the 24 seen during the first test. They were asked to identify those symbols which they had been shown on the first interview. Each symbol was given a "memorability score" that depended on the percentage of participants who correctly identified the symbol as having appeared in the earlier test.

Identical memorability scores were obtained for two of the six test symbols, and these scores exceeded the average for the other 18 symbols tested. Since one of the two also obtained the lowest score in the meaningfulness test, it emerged as the one symbol best qualified as being both unique and memorable (Fig. 1).

Having evolved a suitable symbol, the next step was to attach the desired significance to it. It became important to define as clearly as possible how and under what circumstances the symbol should be used. A use standard was therefore prepared. This standard stipulates that the symbol "shall be used to signify the actual or potential presence of a biohazard and shall identify equipment, containers, rooms, materials, experimental animals, or combinations thereof which contain or are contaminated with viable hazardous agents." It also defines the term "biohazard," for the purpose of the standard, as being: "those infectious agents presenting a risk or potential risk to the well-being of man, either directly through his infection or indirectly through disruption of his environment."

This symbol and the recommendations regarding usage have been submitted to the United States of America Standards Institute for inclusion in their next revision of the "Standard Specifications for Industrial Accident Prevention Signs," Z35.1 code.

This symbol, in fluorescent fire-orange color, has been evaluated during a 6-month period at the National Cancer Institute and other selected laboratories engaged in studies involving hazardous agents. These cooperating research groups included the U.S. Army Biological Laboratories and U.S. Department of Agriculture laboratories, as well as a number of commercial and academic laboratories working under National Institutes of Health research grants and contracts.

In view of its acceptance by the scientists during this evaluation, the National Institutes of Health is recommending that this symbol be used as a general biological hazard warning.

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Note

1. The subject material of this paper was presented at the 6th Annual Technical Meeting of the American Association for Contamination Control, Washington, D.C., 18 May 1967. The work was performed for the National Cancer Institute under contract No. PH 43-65-1045.

7 July 1967

Congenital Malformations Induced by Mescaline, Lysergic Acid Diethylamide, and Bromolysergic Acid in the Hamster

Abstract. *Malformations of the brain, spinal cord, liver, and other viscera; body edema; and localized hemorrhages were found in fetal hamsters from mothers injected subcutaneously with a single dose of mescaline, lysergic acid diethylamide, or 2-bromo-D lysergic acid diethylamide on the 8th day of pregnancy. In addition, all three drugs produced an increase in the percentages of small fetuses per litter, of resorptions, and of fetal mortality.*

As part of (*1, 2*) an investigation of the various possible etiological factors involved in the induction of congenital malformations, I have studied the psychotomimetic alkaloid mescaline (MES), the active principle of the peyote cactus used in the rituals of certain Indian tribes, and the synthetic alkaloid lysergic acid diethylamide (LSD) used on a limited basis in drug therapy of mental disease. In addition, the monobromide derivative of LSD, 2-bromo-D-lysergic acid diethylamide (BOL), was also evaluated.

Pregnant hamsters were obtained from randomly bred stock of Lakeview

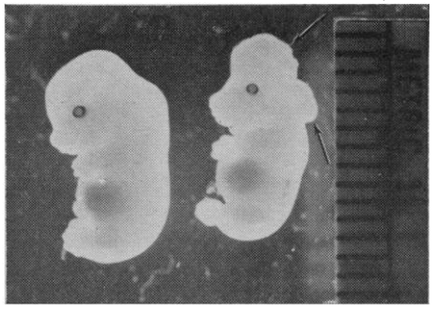

Fig. 1. Normal fetus on left. Fetus on right shows exencephaly. Its mother was injected with LSD (0.003 mg/kg of body weight) on the 8th day of pregnancy.

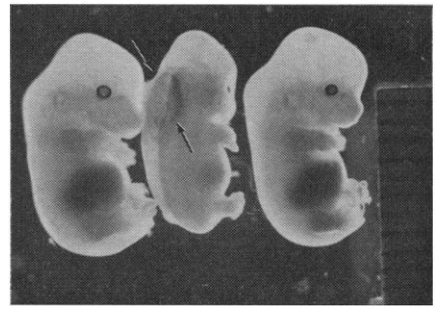

Fig. 2. Middle fetus shows spina bifida. Its mother received mescaline (0.5 mg/kg of body weight) on the 8th day of pregnancy. Normal fetus on either side.

Hamster Colony. All females were caged in air-conditioned quarters maintained at 75°F. Water, Purina laboratory chow, cabbage, and carrots were available ad libitum throughout the experimental period. The animal quarters were illuminated by natural light (roof skylight). Noise was kept to a minimum since this factor has been repeatedly demonstrated to produce deleterious effects in reproductive studies (*2*, *3*).

The compounds were dissolved in sterile saline solution 30 minutes before use and injected (MES, 0.45 to 3.3 mg/kg; LSD, 0.0008 to 0.24 mg/kg; BOL, 0.002 to 0.41 mg/kg) subcutaneously at 1 p.m. on the 8th day of pregnancy. This phase of pregnancy in the hamster is an effective period for the evaluation of the ability of a variety of compounds to cause teratogenesis (*4*). All of the control animals were injected with sterile saline. After the single injection of a drug or saline, the animal was returned to its cage and left undisturbed until the 12th day of gestation, when it was killed by an overdose of ether. As soon as maternal respiration ceased, the uterus was exposed by midline incision, and the number and distribution of fetuses was noted. Each fetus was carefully exposed and examined to determine both its viability and developmental status. The fetus was then placed in 10 percent formaldehyde for 3 days to allow for hardening so that it could be more completely examined for abnormalities.

Table 1. Number and types of fetal abnormalities produced by mescaline, LSD, and BOL. The numbers in parentheses are the average numbers of fetuses.

Drug concentration (mg/kg body wt)	Females (No.)	Fetuses (No.)	Congenital abnormalities (%)	Resorptions (%)	Dead fetuses (%)	Runts (%)
			Mescaline			
0.45	8	86 (10.8)	28	7	5	5
1.33	8	76 (9.5)	9	13	5	0
3.25	8	64 (8.0)	11	25	10	12
			LSD			
0.000084	9	105 (11.6)	6	8	7	7
0.0029	8	86 (10.8)	8	12	15	10
0.021	11	110 (10.0)	5	12	10	9
0.24	9	77 (8.6)	6	14	17	10
			BOL			
0.002	8	87 (11.0)	6	8	3	3
0.025	8	83 (10.4)	7	12	4	1
0.41	7	63 (9.0)	13	22	8	8
			Controls			
Saline (0.9 percent)	25	300 (12.0)	0	2	1	1

There is no major congenital abnormalities were found in the 300 control fetuses, although there was a limited number of runts, dead fetuses, and reabsorbed fetuses found in this group (Table 1). In the experimental fetuses abnormalities of this type were many times greater (by percentage). The types of gross congenital defects found in the experimental litters were exencephaly (Fig. 1), spina bifida (Fig. 2), interparietal meningocele, omphalocele, hydrocephalus, myelocele, edema along spinal axis, edema in various body regions other than spinal area, and hemorrhages of local brain areas (parietal and frontal) and neck (sublingual area). Approximately 10 percent of the abnormal fetuses contained more than one type of defect, and several had four or five defects.

There is no correlation between the dose (milligrams per kilograms of body weight) of the drug administered to the pregnant female and the percentage of congenital malformations found in the fetuses. The lowest concentration of MES actually produced the largest number of defects. However, a dose-response relationship does appear in most cases when the other parameters are considered. The number of fetuses per litter decreased, and the percentage of resorptions, dead fetuses, and runts increased as the concentration of a particular drug administered to the pregnant female was increased.

Recent studies have shown the chromosomal effects of LSD on cultured leukocytes (*5*) and leukocytes obtained from LSD users (*6*). In addition, injections of LSD into rats early in pregnancy has produced runts and increased fetal mortality (*7*).

My results indicate that both lysergic acid diethylamide and its 2-brom derivative can induce a wide variety of congenital malformations in the hamster embryo. Mescaline, although a less potent teratogen as judged by the dose needed to produce anomalies, is nevertheless equipotent in the type and number of abnormalities produced. This difference in potency between mescaline and lysergic acid corresponds qualitatively to the relative psychotomimetic potency of the respective drugs, lysergic acid being the more potent.

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9. I thank Karin Landeen for technical assistance.

7 August 1967

Direction of Translation in Bacteriophage S13

Abstract. *Suppressible* (amber-*type*) *mutants in gene IIIa of bacteriophage S13 show polarity in reducing the activity of only the neighboring gene IIIb. The polarity implies that the genome is translated in the direction of IIIa to IIIb. From the known homology of the messenger RNA with the single-stranded DNA, the orientation of the DNA with respect to the genetic map can also be inferred.*

Bacteriophage S13 is known to have at least seven genes, which have been estimated to account for at least 70 percent of the phage genome (*1*, *2*). The order of these seven genes and the circularity of the S13 genetic map have been established by three-factor crosses (*3*). Data have now been obtained from complementation tests indicating the direction of translation of two of the seven genes. It is inferred that this is the direction of translation of the entire genome.

In the original work (*1*) on complementation in phage S13, the complementation group designated III was considered to consist of two subgroups, IIIa and IIIb, because complementation between suppressible (*su*) mutants in IIIa and IIIb was much lower than complementation between other mutant pairs. However, the discovery of temperature-sensitive (*t*) mutants of group IIIa has led to the finding of strong complementation between *t* mutants of IIIa and *t* mutants of IIIb. A comparison of *su* × *su* tests with *t* × *t* tests is shown in the first part of Table 1. Consequently, IIIa and IIIb are considered to be separate complementation groups. Mutants in IIIb show a markedly altered heat stability of the mature virus, indicating that IIIb determines a viral coat protein (*4*).

Additional complementation tests with both *t* and *su* mutants of groups IIIa and IIIb show that *su* mutants of IIIa are defective in the IIIb function but that *su* mutants in IIIb leave the function of IIIa intact, and *t* mutants in either group have no effect on the function of the other group (Table 1). The table shows that very poor complementation occurs between IIIa and IIIb whenever an *su* mutant in IIIa is used. The difference between *su* and *t* mutants is made more striking by the fact that *t*′43 is a false revertant of *su*43 and therefore both are presumably mutant in the same DNA triplet. These complementation results are qualitatively similar to those observed in bacteriophage T4 (*5*).

The *su* mutants in IIIa are polar, but their polarity does not extend past IIIb into IV. This is inferred from complementation tests between *su* mutants of I, IIIa, IIIb, and IV (Table 2) which show poor complementation only for IIIa × IIIb tests. The gene order is I-IIIa-IIIb-IV (*1*, *3*). The tests in Table 2 were done at 37°C, so the general level of complementation is higher than the tests in Table 1.

All the *su* mutants of S13 are suppressible by one or more of the known *Escherichia coli amber*-suppressor strains such as CR63 and C600, so it is concluded that each *su* mutant contains a nonsense codon and that polypeptide synthesis terminates at the point of the mutation, as has been shown for *amber* nonsense mutants affecting the head protein of bacteriophage T4 (*6*). The chain-terminating property of nonsense mutations is associated with the polar effect observed in genes which

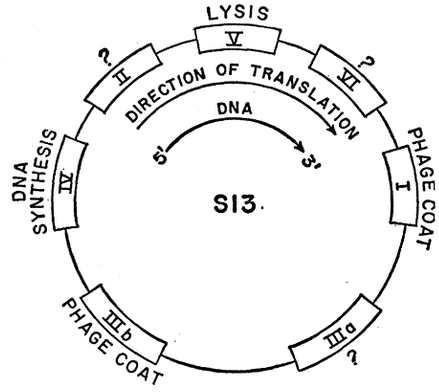

Fig. 1. Map of phage S13 (*1*, *3*) showing the gene functions where known. The direction of translation and the orientation of the single-stranded DNA was inferred from the polar properties of *amber*-type mutants in complementation tests between genes IIIa and IIIb.

normally are coordinately transcribed and translated; there is reduced activity of genes on the side of the nonsense mutation toward which translation would normally proceed (*7*). Accordingly, from the polar properties of the S13 *su* mutants we conclude that IIIa precedes IIIb in the coordinate translation of the two genes, and furthermore that the direction of translation of IIIa and of IIIb relative to the map order is IIIa → IIIb. Moreover, this direction of translation is presumably the same for the entire phage genome (Fig. 1) because of the observed homology (*8*) between the messenger RNA (mRNA) and the mature phage DNA.

It is significant that *su* mutants in other genes are not polar (*1*, *2*, *9*)

Table 1. Complementation tests between *su* and *t* mutants in complementation groups IIIa and IIIb. The tests were done at 42°C except for those marked with an asterisk, which were at 41°C. The method has been described (*1*). *Escherichia coli* C, which does not noticeably suppress any *su* mutant, was used as the host for the mixed infection. The burst sizes of *su* mutants were assayed on the permissive strain *E. coli* C600.1 (*2*), except for tests at 41°C in which cases *Shigella dysenteriae* Y6R (*1*) was used; *t* mutants were assayed on either C, C600.1, or Y6R. All burst sizes were corrected for the burst size of each mutant growing alone under the nonpermissive conditions; this correction produced negative results in two cases. The temperature-sensitive mutant *t*′43 is a false revertant of *su*43.

Mutants crossed IIIa × IIIb	Burst size	Mutants crossed IIIa × IIIb	Burst size	Mutants crossed IIIa × IIIb	Burst size
su × *su*		*su* × *t*		*t* × *su*	
*su*10 × *su*44†	0.6	*su*10 × *t*330	0.8	*t*37 × *su*44†	27
*su*43 × *su*44	0.5	*su*10 × *t*11†	−0.6	*t*37 × *su*66†	40
t × *t*		*su*41 × *t*11†	−3.4	*t*′43 × *su*44†	24
*t*37 × *t*11†	10	*su*43 × *t*11†	4.5	*t*′43 × *su*66†	26
t′43 × *t*11†	10	*su*43 × *t*330	2.0	*t*37 × *su*63*	29
t′43 × *t*330	29	*su*10 × *t*330*	1.1	*t*163 × *su*63*	56
*t*37 × *t*330*	13	*su*43 × *t*330*	2.7	*t*163 × *su*66*	42
*t*163 × *t*330*	37				

* Performed at 41°C and assayed with Y6R when *su* mutants were involved. † Cyanide not used during adsorption.