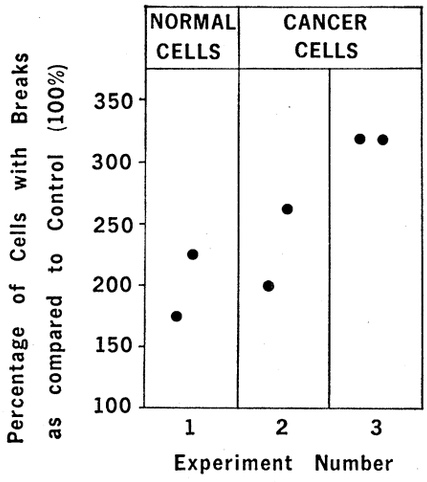

Fig. 3. Cytogenetic effect of cyclamates (200 μg/ml) on human monolayer cultures.

data have been plotted with each control value set at 100 percent (for plotting, the three samples exhibiting no breaks were given values of 1 break per 100 cells), and the results show that, while no clear effect of cyclamates on chromosome breaks at concentrations of 50 to 100 μg/ml can be seen, there is a significant effect at 250 to 500 μg/ml. In contrast to the action of cyclamate, leukocyte cultures supplemented with saccharin (500 μg/ml) showed no evidence of increased breaks.

In a second series of experiments on eight subjects, the procedure was slightly changed. The slides were not flamed during chromosome preparation; coded slides from two experiments were pooled prior to chromosome examination, and approximately 50 metaphases per slide were read (that is, a total of 200 metaphase spreads per culture. Figure 2 shows the results obtained in cultures supplemented with 200 to 500 μg of calcium or sodium cyclamate per milliliter. Chromosome breaks among control cells ranged from 1.5 to 4.5 percent, with an average value of 2.8 percent; in the treated cells, the range was 4.5 to 10.5 percent, with an average value of 6.2 percent. These results correspond well with the doubling found in the first series of experiments. Reported values from different laboratories are 3.7 (*4*), 1.4 (*11*), and 11.7 percent (*12*). Whether the variation in average control values found in the second series of our data, as compared to the first, is due to the smaller numbers of subjects studied, to the procedure of mixing the coded slides from two subjects, to the elimination of flaming the slides, to the greater number of mitotic figures studied, or to a more critical evaluation of breaks is not known.

Figure 3 shows the data obtained in one experiment with monolayer cultures of cells derived from normal human skin cultured in plastic bottles. Two bottles contained sodium cyclamate (200 μg/ml), and two bottles did not receive the supplement. Cells from each bottle were harvested separately, and four chromosome slides were prepared. In the presence of cyclamate, the cells show an approximate doubling of chromosome breaks. Figure 3 also shows the results obtained with cultures of cells derived from human carcinoma of the larynx. Experiment 2 was carried out in a manner similar to that described above; in experiment 3, cultures were made on glass slides in Leighton tubes, and chromosome squashes were prepared. Either way, the data indicate a two- to threefold increase in breakage by the presence of the supplements.

Our results indicate that cyclamate, in a minimum concentration of 200 μg/ml, can stimulate chromosome breakage in human cells in vitro. Whereas a high dosage (equivalent to 15 g/75 kg) was required to obtain a demonstrable increase in chromosome breaks, it should be pointed out that there is some evidence of synergistic actions on chromosome damage between x-irradiation and radiomimetic chemicals (*13*), between the chemical agents and virus (*14*), and between the chemical agents themselves (*15*).

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Hallucinogen-Tranquilizer Interaction: Its Nature

Abstract. *Study of the competition between hallucinogens and tranquilizers at cerebral synapses and on behavior in various species of animals indicates a continuum of effects from protection to dominance of tranquilizer toxicity as the dose of tranquilizer increases. Data on cat and monkey behavior, supplementing that on the rat, show that it is possible to arrive at a tranquilizer dose that can aggravate instead of protect, in accord with the competitive inhibitory nature of the interaction of hallucinogen and tranquilizer.*

Renewed interest in the problem of terminating the action of hallucinogenic drugs, particularly in the so-called "bad trip," has highlighted the necessity for a clearer understanding of the available facts. For example, can one reconcile the clinical accounts of the aggravation by chlorpromazine (CPZ) of the hallucinogenic effects of DOM (4-methyl-2,5-dimethoxy alphamethylphenethylamine) [also known as STP (*1*)], with the failure to obtain other than ameliorative effects in volunteers (*2, 3*)?

It is recognized that overlarge doses of CPZ, instead of allaying symptoms in mentally disturbed patients can, on occasion, themselves induce hallucination (toxic psychosis). Can, then, the therapeutic action, the antidotal action against exogenous hallucinogens, and the hallucinogenic action of large doses of CPZ be harmonized into an expected continuum?

A counterpart and a reasonable answer can be found in experiments with animals. In a previous comparative neuropharmacological survey (*4*) of cerebral synaptic transmission conducted by monitoring the output potentials evoked by a constant, submaximum input (*5*), we showed that the

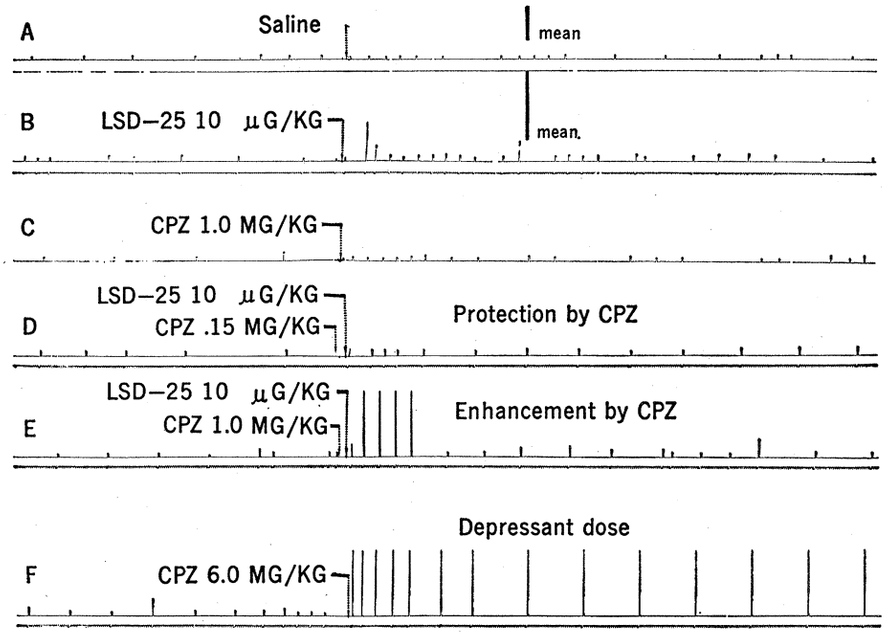

Fig. 1. Effect of CPZ-LSD interaction on conditioned approach for food in spider monkey on a regular reinforcement schedule controlled by a 2-second signal tone lasting 20 seconds. Time was marked every 30 seconds. Conditioned stimulus was marked by the same pen. Means were omitted for the large interaction effects. Drugs were given intraperitoneally (in sessions 14 or more days apart) immediately after control recordings. Controls by injection with saline were obtained on adjacent days.

impediment to transmission characteristically induced by lysergic acid diethylamide (LSD) and other catechol and indoleamine hallucinogens can be characteristically prevented by low doses of CPZ and aggravated by larger doses (*6*). In fact, large enough doses of CPZ can produce synaptic depression by themselves, so that the impediment of transmission associated with the action of hallucinogens is also a property of large doses of tranquilizers. Indeed, the magnitude of the ratio of depressant to protective dose (a form of therapeutic ratio), is 20 for CPZ, but it is 1 or less for simple sedatives like phenobarbital (see *7*); this ratio provides a numerical expression and a definition of the selectivity that distinguishes the tranquilizers from simple sedatives, and it also is an expression of central "side effect."

The data on animal behavior corresponds. In rats (*8*) conditioned by water reward to respond to a tone, CPZ in smaller doses protects against the delay in response induced by LSD (*9*). Given alone in larger doses, CPZ impairs behavior, causing a delay in response indistinguishable from that produced by LSD and other catechol and indoleamine hallucinogens. In keeping with this, some intermediate doses of CPZ, though not depressant in themselves, when given with hallucinogens intensify rather than decrease the LSD effect. The corresponding continuum of effects with increasing CPZ doses is shown in the spider monkey and in the cat. The CPZ was initially overtly ineffective alone but was protective against LSD; it was then additive with LSD; and finally it was depressing alone.

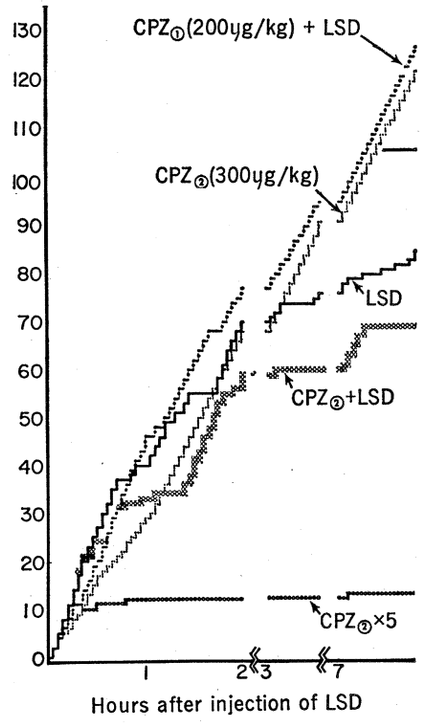

Fig. 2. Effect of CPZ-LSD interaction on differential approach for milk in cat. Stimuli: correct or discriminative (S_D), 2 khz; incorrect and therefore nonreinforced (S_Δ), 1 khz tones. Stimulus interval 1.5 minutes and duration 30 seconds. Maximum number of responses per stimulus was four. Drugs were given intraperitoneally in sessions 14 or more days apart. The "protection" curve has the same slope as that obtained with saline (control) obtained on the preceding day (but not shown). LSD was injected intraperitoneally at zero time; CPZ was given intraperitoneally 30 minutes before zero time.

Unrestrained spider monkeys, maintained at 80 percent of normal body weight by restricting food, were rewarded with a food pellet every time they pressed a lever in response to a 2-khz tone. The response latencies of one of these monkeys, typical for the five studied, are shown in Fig. 1. The response latencies are the same whether saline or CPZ (1.0 mg/kg) is given (Fig. 1, A and C). The LSD increases the response latencies (doubles the mean) (Fig. 1B). This effect is prevented by 0.15 mg of CPZ per kilogram (Fig. 1D), but is intensified by 1.0 mg of CPZ per kilogram (Fig. 1E); whereas 6.0 mg of CPZ per kilogram by itself increases the response latencies (Fig. 1F).

Similarly cats, with food intake restricted to 70 percent of satiating amounts, were rewarded with 1 ml of milk when they discriminated between a 2 khz (correct) and a 1 khz (incorrect) tone by interrupting a light beam in response to the 2 khz tone. The cumulative records of one cat, typical of the three studied, are shown in Fig. 2. Only the correct responses are shown. The solid curve traces the reduction due to LSD, whereas the top curve, which is identical to that for saline alone (not shown), indicates the protection afforded by prior injection of CPZ (200 μg/kg). The next curve, with no change in slope, shows the lack of overt action of a larger (300 μg/kg) dose given alone, while the hatched curve shows that this larger dose adds to the LSD depression so that it now exceeds that of LSD alone. The bottom curve shows that CPZ given alone in sufficiently large doses produces a depression.

The data consistently indicate that one can select from the continuum of tranquilizer actions, by appropriate dosages, both protective and enhancing actions. An extrapolation to man sug-

gests the explanation of a potential action of tranquilizers in enhancing hallucination when tranquilizers are administered in the right dosage range. Finding only a protective action (*2*) would be in agreement with the interpretation that an additive effect is an aspect of the competition for receptors between LSD and CPZ that requires a special ratio of one to the other. As already noted, the "toxic psychosis" from large doses of CPZ is consistent with the interpretation. Although the alleged experience of the drug abusers suffers from the vagaries of drug contamination and concomitant use of additional drugs, it does suggest that occasional fortuitous arrival at just the right ratio of hallucinogen to tranquilizer might indeed worsen matters. Trials of DOM in animals and man should provide a further test of this concept. In man one can measure the dissociation underlying hallucinogenic action (*10*) by quantitative, instrumental recording of perceptual changes resulting from subclinical test doses producing no symptoms (*11*).

The accumulated data show that a tranquilizer acts as weak psychotogen protecting against a stronger one by substituting for it at receptor sites, but in large enough doses adding to or even producing the effect it was intended to correct. This accounts for the reported occasional aggravation of hallucinogenic action by tranquilizers.

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Glycine in the Spinal Cord of Cats with Local Tetanus Rigidity

Abstract. *In cats, significant loss of glycine occurred in spinal grey matter on the side of local tetanus, whereas the γ-aminobutyric acid concentration remained unaltered. These findings suggest that tetanus rigidity is due to the blocking of the spinal inhibitory transmission by decrease of inhibitory transmitter and that glycine is an effective inhibitory transmitter in cat spinal cord.*

The possibility that amino acids may act as synaptic transmitters has been demonstrated for invertebrates (*1*). However, little is known about the excitatory and inhibitory transmitters in the mammalian central nervous system.

Glycine may be an inhibitory transmitter involved at synapses in cat spinal cord (*2–4*), and γ-aminobutyric acid (GABA) may be an inhibitory transmitter released from neurons located in the rostral level of central nervous system in vertebrates (*5*). An injection of tetanus toxin in one extremity of cats produces a strictly localized rigidity, which has been called local tetanus. It is also clear that the action of tetanus toxin is at inhibitory synapses on motoneurons (*6*). We have studied local tetanus rigidity in connection with the chemical transmitter involved. Tetanus toxin [10×10^4 MLD (minimum lethal doses for mice) in 0.1 ml] was injected into the right gastrocnemius muscle of mature cats. Symptoms of local tetanus appeared in the injected muscle in 24 hours, and rigidity involved the whole right lower extremity in 72 hours. The left lower and the upper extremities remained unaffected. With nembutal anesthesia, laminectomy of the lower lumbar and the upper sacral vertebrae was carried out.

The sixth and seventh lumbar segments and first sacral segment of the cord were removed, rinsed in saline, blotted, and frozen on dry ice. Thin sections (2 to 3 mm) of the cord were cut and divided into the grey matter and white matter on the control and the local tetanus sides, respectively. All dissections were performed at −20°C on an aluminum plate on which dry ice bags containing acetone were placed.

Table 1. Contents of glycine, GABA, and total amino acids in the right side with local tetanus and in the left control side of cat spinal cord. Student's t test in paired experiments was used to test the data. $t_{(N-1)} = \bar{d}/S\bar{d}$; where $\bar{d}$ is the mean of the difference in each experiment, $S\bar{d}$ is the standard error, and N is the number of experiments. N.S., not significant.

Case No.	Grey matter (μmole/g)			White matter (μmole/g)		
	Control side (L)	Local tetanus side (R)	Difference	Control side (L)	Local tetanus side (R)	Difference
			Glycine			
1	5.7	4.6	− 1.1	3.0	3.6	+ 0.6
3	6.1	5.9	− 0.2	3.2	2.7	− 0.5
4	5.6	5.3	− 0.3	2.7	2.9	+ 0.2
5	6.1	5.8	− 0.3	3.3	3.4	+ 0.1
6	6.7	6.6	− 0.1	2.9	3.8	+ 0.9
7	5.4	5.3	− 0.1	3.0	2.8	− 0.2
8	5.9	5.5	− 0.4	3.3	3.1	− 0.2
18	5.9	5.4	− 0.5	3.9	3.5	− 0.4
19	5.7	5.2	− 0.5	2.8	3.6	+ 0.8
20	4.8	4.4	− 0.4	2.6	2.6	0.0
21	5.8	4.9	− 0.8	2.7	3.2	+ 0.5
			$P < .005$			N.S.
			GABA			
1	1.00	0.81	− 0.19	0.10	0.05	− 0.05
9	1.46	1.69	+ 0.23	0.18	0.24	+ 0.06
10	1.57	1.18	− 0.39	0.15	0.12	− 0.03
11	1.04	1.27	+ 0.23	0.17	0.13	− 0.04
12	0.98	1.12	+ 0.14	0.22	0.26	+ 0.04
13	0.88	0.62	− 0.26	0.14	0.15	+ 0.01
19	0.77	0.79	+ 0.02	0.11	0.15	+ 0.04
20	1.24	1.41	+ 0.17	0.14	0.14	0.00
			N.S.			N.S.
			Total amino acids			
3	30.3	31.3	+ 1.0	22.9	18.6	− 4.3
4	33.0	32.4	− 0.6	24.7	25.0	+ 0.3
6	34.6	34.6	0.0	24.6	26.4	+ 1.8
7	33.2	31.8	− 1.4	24.2	23.5	− 0.7
8	33.2	33.8	+ 0.6	24.0	24.3	+ 0.3
12	35.0	34.1	− 0.9	20.2	18.3	− 1.9
13	33.0	32.9	− 0.1	20.9	16.2	− 4.7
18	35.0	38.0	+ 3.0	19.7	19.7	0.0
19	38.0	35.0	− 3.0	17.4	20.0	+ 2.6
20	38.5	36.0	− 2.5	24.2	22.1	− 2.1
			N.S.			N.S.