graphs demonstrated that, in a culture of ts-546 cells at 39° C, the number of round, refractile, mitotic-like cells increased continuously and subsequently floated into the medium. When a culture of ts-546 cells was incubated at 39° C for 24 h, at least 50% of the cells appeared to be rounded and mitotic-like. At the same time, few dumbbell-like figures characteristic of anaphase or telophase cells were seen (Fig. 5).

The number of metaphases and ts-metaphases increased from approximately 50% of the mitotic figures at the time of temperature switch to close to 100% 8 h later (Fig. 4*b*). The number of anaphases and telophases correspondingly decreased from 50% to nearly 0.

The results show that when hamster ts-546 mutant cells were switched from 33° to 39° C, the cells ceased to divide almost immediately. With further incubation at 39° C, cells were killed regardless of their density. Reversion frequency was about 5×10^{-7}. No generalised defect in DNA, RNA or protein synthesis was found at the non-permissive temperature. At 39° C the mitotic cells were arrested in metaphase and ts-metaphase, which is seen as a failure of the normal spindle fibre mechanism to hold the chromosomes in their normal metaphase orientation. The ts-metaphase figures, under a light microscope, appear to be similar to the c-metaphases resulting from colchicine treatment.

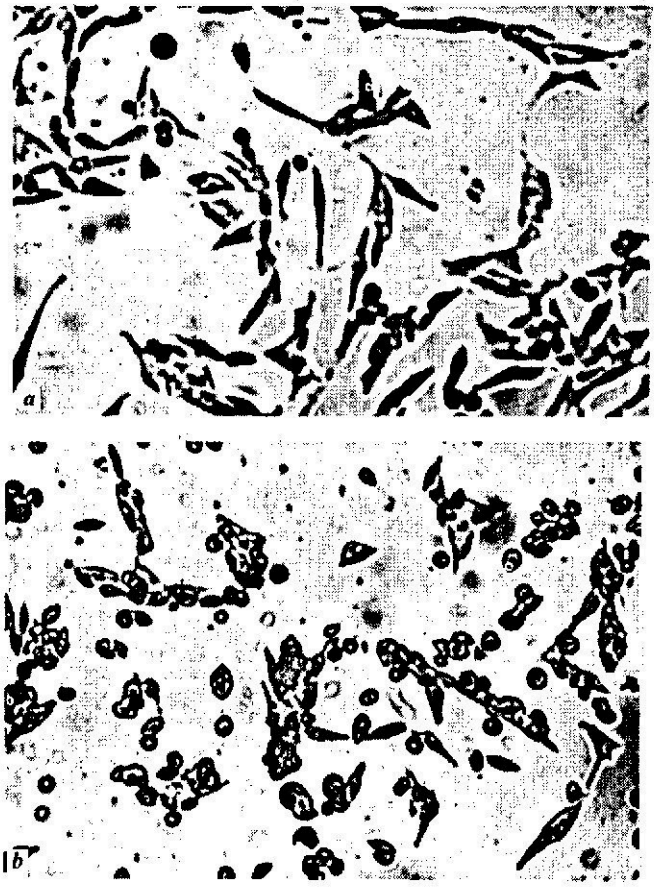

Fig. 5 Accumulation of mitotic-like cells at 39° C. Cells in dishes in log-phase grown at 33° C were incubated at 39° C for 24 h when the photograph was taken. (×140.)

Since mitotic cells generally synthesize very little or no RNA[12], one may ask that why is no reduction of RNA synthesis observed at 39° C? The cells used for measuring ^{3}H-uridine incorporation were those which remained attached to the dishes where the maximum number of mitotic cells reached only 8% after shifting to 39° C (Fig. 4*a*). Consequently 92% or more of the cells used in the incorporation experiment were non-mitotic. The finding that the cells not in mitosis incorporate a normal amount of ^{3}H-uridine is thus compatible with the conclusion that the ts-546 cells in mitosis are blocked in metaphase at the non-permissive temperature.

Several mechanisms might account for the accumulation of mitotic cells in metaphase and ts-metaphase at 39° C. The gene controlling the production of spindle fibre proteins or microtubules could have suffered a mutation resulting in defectiveness and inability of the proteins or microtubules to orient properly at 38° C. A second possibility is that a protein holding the spindles together became defective. A third alternative is that a protein holds the spindle proteins and chromosomes together, becoming defective at the non-permissive temperature, so that the chromosomes are disengaged from the spindles. It is also conceivable that the defective protein, when normal, binds the spindles to the centriole. There is also the possibility that the synthesis of the spindle fibre proteins or other factors involved in karyokinesis failed to take place at 39° C. These possibilities are being investigated.

It is to be hoped that the isolation of temperature sensitive mutants defective in mitosis could lead to the identification of factors which are essential in mitosis. It might also be possible to identify the time during the cell cycle when these factors are synthesised. Furthermore, the possibility that mitosis can be arrested because specific factors become defective at a higher, non-permissive temperature may provide methods of arresting metaphase and controlling cell growth by specific inhibition of these factors.

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Facilitation of Learned Resistance to Audiogenic Seizures in Balb/cCrgl Mice by *d*-LSD-25

ALTHOUGH conditioning techniques applied to electrophysiological behaviour are presently being used in behaviour therapy for human epilepsy[1,2], complete cure is still a hope of the future. Extensive reports indicate that minute amounts of *d*-lysergic acid diethyl amide (*d*-LSD-25) could be used to assist current biofeedback training and other therapies used to effect change in the EEG and seizure susceptibility of

epileptics[3] and we note many studies of the facilitation effect of small doses of *d*-LSD-25 on learning and memory[4–13].

Several methods, including many behavioural ones[14–18], are known for changing audiogenic seizure susceptibility in inbred mice. Elaboration of a conditioned response is known to interfere with the occurrence of these seizures[19] and exploratory activity and learning ability are positively correlated with seisure resistance in rodents[20], as are several factors related to stress[14,21]. We confirmed these effects in experiments with mice in which the administration of 3.65 μg of *d*-LSD-25, 30 min before conditioning, significantly facilitated long-term habituation and also conditioned inhibition of audiogenic seizures induced by an intense, high-frequency sound presented 1 d after the end of training.

Long-term habituation was brought about by exposing the mice to a subseizure threshold tone 1–4 d before challenging them to seize with the louder tone. This effect is related to the classical 48 h refractory period found after intense stimulation[14]. Conditioned inhibition of seizures was accomplished by pairing a light with brief subthreshold tones, and subsequently presenting the light 5 s before the challenge stimulus on test night.

The use of multiple exposures to improve estimates of seizure susceptibility was first reported by Fuller and Sjursen[22]. Henry[23] later showed that a priming stimulation of only 30 s at an early age induced development of seizure susceptibility which peaked 4–5 d later, suggesting a method of more reliable control of seizure susceptibility. In standardising procedures for Balb/cCrgl mice, we found that peak seizure incidence (88%) on day 26 after priming on day 21 was equal to the cumulative seizure incidence computed as the percentage of mice that seized at least once during four consecutive exposures on days 21, 28, 35 and 42. In our experiments, the treatment variables were introduced between days 21 and 26.

Six hundred and eighty-five mice were randomly assigned to groups by split litter design, weighed and used to test the effects of pure *d*-LSD-25 (Sandoz) given intraperitoneally (7.3 μg ml^{-1}; average dosage 180 μg kg^{-1}; standard dose, 3.65 μg per mouse) on the alteration of audiogenic seizure susceptibility by experimental and control procedures which are listed below. All mice were primed on day 21 (1900 h) and tested on day 26 (1900 h). The main experimental procedures were as follows. Series 1, running to escape from the hand of the experimenter in an activity wheel at 50 m d^{-1} on days 22–26 (1900 h); series 2, the direct effect of *d*-LSD-25 given on days 21 and 26 (30 min before priming and testing respectively) as compared with the same injected only on days 24 and 25 (1900 h); series 3, the effect of *d*-LSD-25 given 30 min before training on long-term habituation on test night induced by five 1 min alternating exposures to a subseizure threshold 10 kHz pure tone (60 dB above 0.0002 dynes cm^{-2} presented repeatedly on days 22–25 (1200 h) as compared with the effect when injected 30 min before receiving 20 min continuous exposure on days 24 and 25 (1200 h); series 4, the effect of *d*-LSD-25 injected 30 min before a 15 min non-random classical conditioning session of delayed light (25 W red, diffuse bulb)–tone (10 kHz at 60 dB above 0.0002 dynes cm^{-2}) pairing (ISI, 1.5 s, US, 10 s, time-out, 15 s) on day 25 (1900 h). On day 26 the conditioned stimulus (red light) was presented 5 s before the challenge stimulus (condition CS in series 4 of Table 1).

Drug controls received equal amounts of handling and of the diluent (0.9% sterile saline) and yoke stimulation. Additional control groups were included as indicated in Table 1. For series 1, controls received equal amounts of exposure to the hand of the experimenter in a fixed activity wheel, in series 2, controls were not primed, and in series 3 they received equal exposure to the experimental chamber. For series 4, experiment 1, controls received equal cyclic exposure to light-only and tone-only. Controls in experiment 2 of series 4 received identical light–tone pairing but were not exposed to the conditioned stimulus on day 26 during challenging (Conditions CS).

Table 1 Effect of *d*-LSD-25 and learned resistance to audiogenic seizures in Balb/cCrgl mice

Series	Experiment	Condition	Drug	% Seized	N	adj. χ^2
1		Activity wheel training				
		Run	—	62	37	
		Yoke	—	86	36	
			Total		73	4.60*
2		Direct action of LSD				
	1	Primed	LSD	88	16	
			Saline	75	16	
		Unprimed	LSD	0	16	
			Saline	0	16	
			Total		64	0.59
	2	Primed	LSD	84	25	
			Saline	92	25	
			Total		50	0.09
3		Habituation and LSD				
	1	Tone	LSD	50	50	
			Saline	84	50	
		Controls	LSD	90	10	
			Saline	90	10	
			Total		120	13.02†
	2	Tone	LSD	0	20	
			Saline	25	20	
		Controls	LSD	65	20	
			Saline	85	20	
			Total		80	27.73†
4		Conditioned inhibition and LSD‡				
	1	Light–tone	LSD	41	37	
			Saline	60	37	
		Light–only	LSD	66	37	
			Saline	66	37	
		Tone–only	LSD	60	25	
			Saline	60	25	
			Total		198	6.19
	2	CS	LSD	28	25	
			Saline	44	25	
		$\overline{CS}$	LSD	52	25	
			Saline	56	25	
			Total		100	4.43

* $P<0.05$.
† $P<0.001$.
‡ LSD, light–tone treatment is significantly different from remaining control groups pooled in this series (adj. $\chi^2=5.31$, d.f.$=1$, $P<0.05$).

All mice were primed and tested with a 90 s 10 kHz pure tone at 100 dB above 0.0002 dynes cm^{-2}. A concentration check after 6 months showed no change in the LSD solution kept in the dark, near room temperature.

The results are summarised in Table 1. Repeated locomotor practice significantly reduce seizure susceptibility by 24%. This effect is not strong and was not significant in a smaller sample (data included in experiment 1 of series 3) treated with *d*-LSD-25. The effect is not due to a single session of running just before testing on day 26. Earlier work with activity wheel stress and audiogenic seizures found very similar results[15].

At 3.65 μg per mouse, repeated or single doses of *d*-LSD-25 had no direct effect upon seizure susceptibility. Only one 3.65 μg dose, however, increased conditioned inhibition and long-term habituation of audiogenic seizures in the presence of the conditioned-unconditioned and unconditioned stimulus respectively. The increase in habituation was more significant. Future work on conditioned inhibition might use more elaborate procedures to study the effects of *d*-LSD-25 on higher learning.

These results did not depend on *d*-LSD-25 being present during testing performed 24 h after injection and training. Controls showed learning occurring to a lesser degree independent of the *d*-LSD-25 state during acquisition. These results support earlier findings that low amounts of *d*-LSD-25 facilitate ongoing processes in the brain, affecting learning and memory[1-19]. Studies of the best time relationships between states and of the use of equal or preferably smaller doses to effect reverse tolerance are needed. The symptoms of overdose are well known to have effects opposite to those of lower doses[3].

Mice exhibit the highest resistance to high-dose effects of *d*-LSD-25, as much as 300 μg per mouse being required to produce overt changes in its behaviour[24]. We have shown here that as little as 1% of this dose caused significant facilitation of learned behaviour which reduced subsequent seizure susceptibility. Although caution must be observed[25] these data may be extrapolated to the human condition on at least three grounds. Similar forms of reflex epilepsy exist between the two species (for example, startle, audiogenic and musicogenic epilepsy[26-29]). Acoustic stimulation has been found to be superior to intermittent photic stimulation or hyperventilation in defining the cortical paroxysmal focus in the EEG of humans with temporal lobe epilepsy[30]. (There is reason to believe that a cortical focus facilitates the audiogenic seizure in mice.) Also, seizures in both species are subject to the effects of learning[1-3,19].

The facilitation effect of single low doses on learning has been reported for humans as well as several animal species in many different situations. Suggestions that *d*-LSD-25 causes undesirable side-effects, such as chromosomal damage, when used in clinically useful doses, have been refuted by investigations *in vitro* and *in vivo* in several species including humans[3,31-37]. As neural degeneration and functional depression due to anticonvulsant medication are a problem[38-40], human experimentation with low dose schedules and learning should be considered.

By comparing dose-effect data from the mouse and human it is possible to estimate the optimum dosage required to facilitate learning and memory in the human. The mouse detoxifies *d*-LSD-25 ten times faster, has a metabolic rate ten times greater and a much lower brain/body weight ratio than humans; so its high resistance to direct effects is not surprising. Overdose symptoms peak at approximately 300 μg for the mouse[24] and 1,000 μg for the human[41]. As 1% of this dose significantly reduced seizure susceptibility in mice through learning, the optimum dose facilitating condition inhibition of seizures in human epileptics could be as low as 10 μg or less (that is, about 0.1 μg kg^{-1} as compared with the so-called moderate dosage of 1–2 μg kg^{-1}). Further, since 100 μg represents the anticonvulsant threshold in mice, at least 300 μg should be required to suppress motor seizures directly in the human epileptic.

The physiological mechanisms underlying the effect of *d*-LSD-25 on brain function are relatively well understood[3]. It acts directly as an anticonvulsant when taken in high doses. Approximately 1–5 mg kg^{-1} must be given to the mouse to increase seizure resistance independent of conditioning[42,43]. This direct protection is related to the facilitation of motor and sympathetic nervous activity within this dose range for the mouse[42,44-47]. High dose inhibition of synaptic transmission through the thalamus[48] is probably not a necessary part of the anticonvulsant action in mice, since all brain rostral to the inferior colliculus is unnecessary for elaboration of the audiogenic seizure sequence[49], but may be where subcortical activation of a cortical focus is necessary for the seizure to occur[50,51].

Bradley and coworkers[52] showed that small amounts of *d*-LSD-25 amplify the ongoing effects of sensory collateral inputs to the reticular formation of the brainstem without directly affecting sensory input to the cortex[53] or directly exciting the output cell core[54] of the reticular formation. They later discovered that these effects focused attention of the animal on to a neutral or habituated stimulus to the degree found with conditioned stimuli (where response thresholds were not affected by the dosage used) without directly arousing the animal or preventing its sleep. They suggested that in small amounts, *d*-LSD-25 increases the significance level of sensory stimuli by facilitating mechanisms which underlie attention and block short-term habituation. The inhibitory effects of attentional mechanisms in animal and human epileptics are extensively documented although they are still not fully understood[55].

Amphetamine has also been shown to facilitate learning and memory only within an optimal dose range[56] and also to reduce audiogenic seizure susceptibility at high doses[21,42,43]. An optimal range in the dose-effect curve is a classical finding. Some drugs that facilitate learning at low doses, however, act as poisons and convulsants at higher ones (for example, strychnine)[56]. Drugs chosen to facilitate behaviour therapy for epilepsy could conveniently act as anticonvulsants at high or cumulative doses while simultaneously minimising side-effects.

A recent review[57] re-affirmed past conclusions that LSD can be a safe therapeutic instrument when properly administered to suitable subjects in controlled settings. Tetrahydrocannabinol[58,59,60] and psilocybin[50,51] also act as anticonvulsants. This combination of facilitation of learning and memory in minute doses, direct anticonvulsant action in the upper dose range, reverse tolerance with proper scheduling, and the absence of addiction and proven side effects directly attributable to the drugs, suggests the use of psychedelic compounds as effective and safe adjuncts to current behavioural and electrophysiological biofeedback procedures presently used to treat epilepsy and a variety of other illnesses.

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Augmentation of Cytotoxic Drug Action by Antibodies Directed at Cell Surface

THE concept of attaching cytotoxic agents to tumour-specific antibodies in the treatment of cancer is therapeutically attractive. In this way the specificity of the agents would be increased and their systemic toxicity reduced. This immunochemotherapeutic approach has been successfully applied in experimental systems when the cytotoxic agent has been covalently bound to the antibody[1-3]. Similar success has been reported with the nitrogen mustard chlorambucil, merely physically adsorbed on to the globulin fraction from tumour-specific antiserum without the actual formation of a covalent bond[4]. It is surprising that antibody could act as a carrier for chlorambucil under these conditions, as has been proposed[5], as the two molecules would be expected to dissociate in the *in vivo* environment. It is, therefore, probable that the increased cytotoxicity of chlorambucil in the presence of antibody is attributable to some other mechanism. The experiments described here are an attempt to elucidate this problem further.

The target cells for these experiments were polyoma-transformed BHK21/C13 cells (J_1) grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum which had been heated to 56° C for 30 min to inactivate complement. The cells were collected using a Tris-buffered saline containing 0.02% versene. An antiserum to these cells was produced in New Zealand white rabbits. Each rabbit received approximately 1×10^8 intact cells intramuscularly twice weekly for 4 week. The resulting antiserum contained antibodies for surface components of J_1 cells as shown by membrane immunofluorescence by the indirect method using fluorescein-labelled sheep antirabbit globulin (Miles-Serevac). The antiserum was also cytotoxic for J_1 cells in the presence of guinea pig complement (Wellcome) as shown by isotope release from cells prelabelled with radioactive chromium (^{51}Cr); 50% of cells were lysed at a 1 : 320 dilution. Control normal rabbit serum (NRS) was obtained by bleeding the rabbits before immunisation. Both the NRS and the immune serum (IRS) were heat inactivated, sterilised through a 0.22 μ Millipore filter and stored

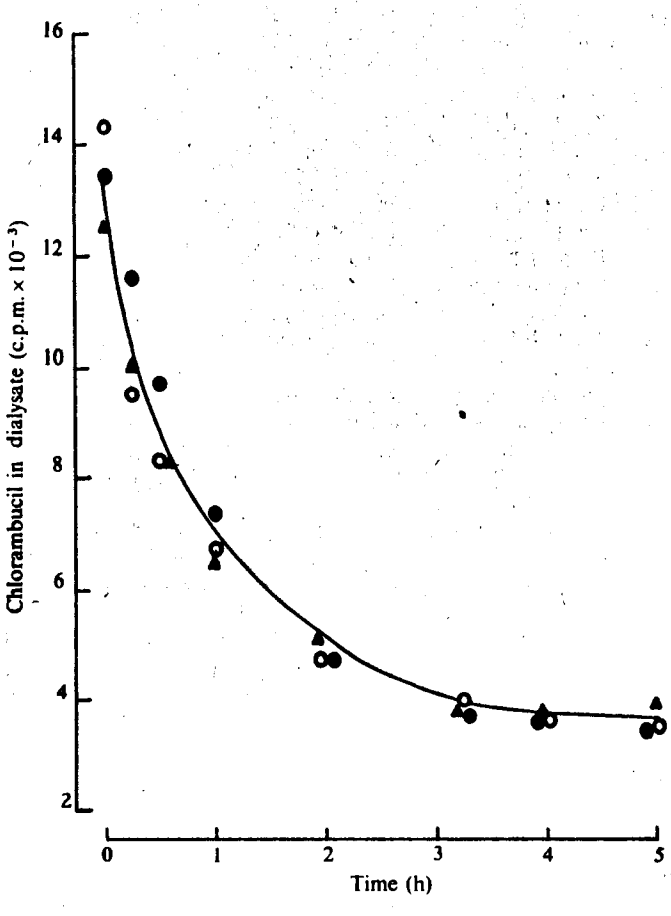

Fig. 1 10 ml volumes of undiluted calf serum were placed in lengths of Visking dialysis tubing (0.25 inch diameter), which were then arranged as flat coils and covered with 10 ml of one of the following solutions: (1) 1×10^{-6} M ^{3}H-chlorambucil (specific activity 118 mCi mmol^{-1}) in PBS (○): (2) rabbit immunoglobulin (IgG) (Miles-Serevac) 50 μg ml^{-1} with 1×10^{-6} M ^{3}H-chlorambucil preincubated on ice for 15 min (▲); (3) bovine serum albumin 50 μg ml^{-1} with 1×10^{-6} M ^{3}H-chlorambucil preincubated on ice for 15 min (●). Dialysis proceeded at 37° C and 100 μl samples of the dialysate were taken at timed intervals for scintillation counting.