and *Escherichia coli* QM B1457), yeasts (*Saccharomyces cerevisiae* ATCC 7753), actinomycetes (*Streptomyces aureofaciens* ATCC 10762), and higher fungi including *Mucor murorum* QM 776, *Penicillium chrysogenum* Wisc. Q176, and *Neurospora crassa* ATCC 9683. Additional experiments are in progress. It is hoped that this technique may be used to study hyphal fusion, cytoplasmic inheritance, and genetic recombination as well as the more common developmental processes. Other applications may be expected to become apparent as the technique is explored further.

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A Quantifiable Behavioral Correlate of Psychotogen and Tranquilizer Actions

Abstract. A representative psychotogen, lysergic acid diethylamide (LSD-25), in doses small enough to be devoid of gross effects, increases response latency in rats to a tone indicating the availability of water reward; this effect is greatly reduced by prophylactic administration of a representative phenothiazine tranquilizer, chlorpromazine (CPZ), in doses that per se do not affect performance. The nature of the chlorpromazine action and its competition with lysergic acid diethylamide is revealed by the effects of chlorpromazine in larger doses.

It was felt that the significance of the cerebral synaptic inhibitory action of psychotogens and the competition by tranquilizers established in these laboratories (*1*) could be understood further and a beginning made in assessing their role in behavior, if quantitative behavioral correlates could be found. One correlate that has proved valuable has been the lever-pressing of thirsty rats for a water reward, when a tone indicates its availability. This conditioned approach behavior, monitored by measuring the stimulus-response latency (length of upper vertical line, with corresponding reward achievement indicated by the marks on the lower line of each strip in Figs. 1 and 2), shows clear inhibition after intraperitoneal injection of a moderate dose of lysergic acid diethylamide (strip 3). As seen in an example (Fig. 1), the latency exceeds the 20-second period within which reward is possible and recycling of the trial takes place after a randomly timed interval. In the doses used, the inhibitory action is selective and not due to general sedation. A preventive intraperitoneal injection of chlorpromazine, in a dose that per se produces no change in the same animal (strip 2), readily gives protection as shown by the abbreviated effect of subsequent lysergic acid diethylamide (strip 4) (*2*).

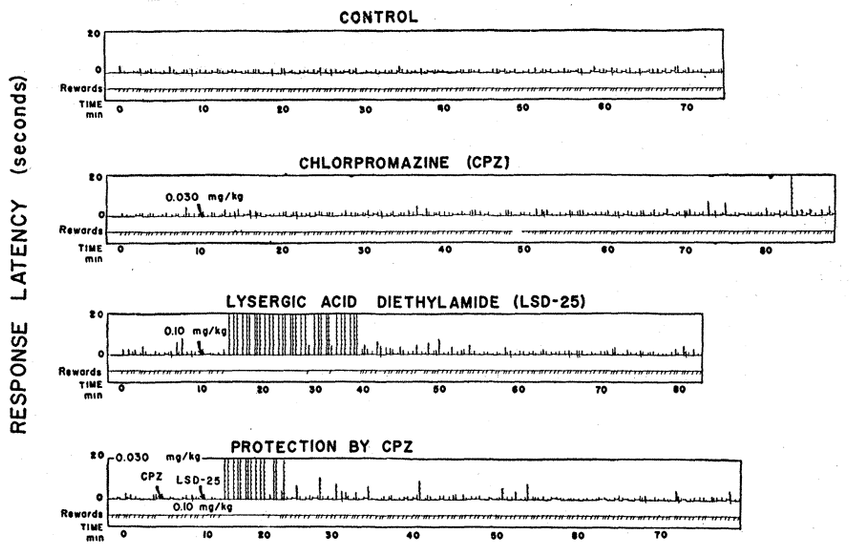

Fig. 1. Lysergic acid diethylamide (LSD-25) inhibition and chlorpromazine (CPZ) protection on approach behavior in rat.

The nature of the antagonism of lysergic acid diethylamide and chlorpromazine to each other is revealed by the effects of larger doses (Fig. 2). Thus, the larger, but nondepressant dose of chlorpromazine enhances instead of reducing lysergic acid diethylamide inhibition, while a still larger dose produces a depression of approach behavior that resembles the effect of lysergic acid diethylamide, but is distinguished from it by a considerable degree of sedation. The observed antagonism and summation are the characteristics that mark the competition between two substances with like actions but very different potencies. Because of this the weaker agent can, when it has gained access to the site of action (receptor) in moderate quan-

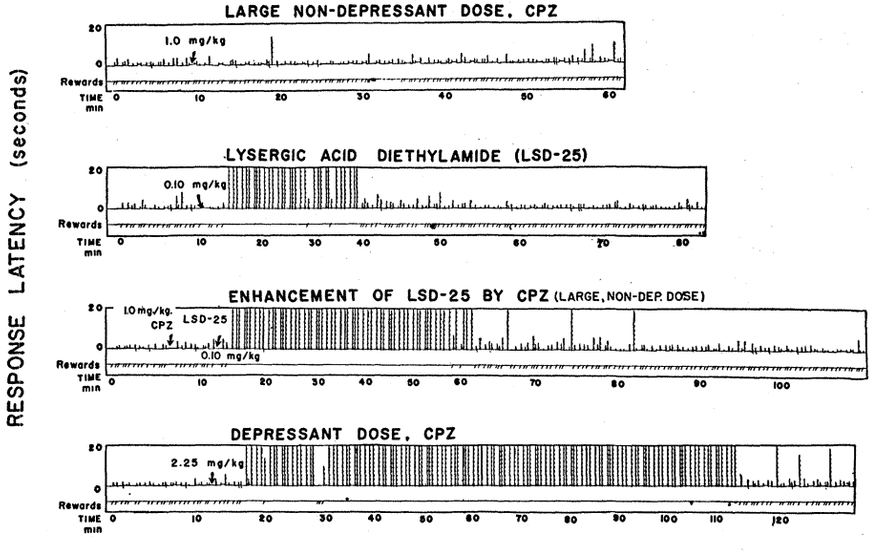

Fig. 2. Effects of larger than protective doses of chlorpromazine (CPZ) on approach behavior in rat.

tities, pre-empt a portion and, by substituting a weak for a strong action, subtract from and compete with the stronger action.

The parallelism between cerebral synaptic and behavioral actions, including reported clinical effects, of lysergic acid diethylamide and chlorpromazine and their competition lends support to the interpretation and confidence in the methods utilized.

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2. The effects of the injection procedure per se and of possible local actions at the site of injection were excluded by negative results with saline injection and with injections of LSD-25 during periods of high tolerance to this drug. The latter helps eliminate the possibility that a local irritant effect of LSD-25 might have been thought to be offset by an alleged local anesthetic action of chlorpromazine.

21 November 1960

Action of d-Tubocurarine Chloride on Net Flux of Water across Isolated Frog Skin

Abstract. *d*-Tubocurarine chloride, when added to the solution bathing the outside of the isolated frog skin, enhances the net flux of water which arises from the influence of an osmotic gradient. Although this effect appears to result from alteration in the pore size of the membrane, it is not accompanied by any consistent change in the resting potential.

Some years ago, Kirschner (*1*) showed that the addition of *d*-tubocurarine chloride (curare) to the solution bathing the outside of the skin of various species of frogs produces a reversible increase in the active transport of sodium. These results have been confirmed by others who observed a similar effect with a variety of neurotropic compounds (*2*). On the other hand, the lack of response of the skin of *Rana temporaria* L. to curare, already noted by Kirschner, was also confirmed and shown to result from hormonal variation (*3*).

In order to explain the enhancement of the active transport of sodium, it has been proposed that curare acts by increasing the passive permeability to sodium of the membrane of the skin epithelial cells which face the outside. This results in an increase in intracellular sodium concentration, which in turn stimulates the active transport mechanism for sodium. One way in which the passive permeability may be increased is by changing the pore size of the membrane. If this hypothesis is correct, one should also expect a modification of the net flux of water arising across the frog skin under the influence of an osmotic gradient. The purpose of the present study has been to test this hypothesis by measuring the net flux of water across the skin and to determine the effect of curare on such flux.

The apparatus used is similar in principle to the one described by Koefoed-Johnsen *et al.* (*4*). The experiments were performed on the isolated skin of *Rana temporaria temporaria* L. bathed with ordinary Ringer's solution on the inside and with Ringer's at a 10-fold dilution on the outside. After a control period of about 4 hours, curare was added to the outside solution at a concentration of 170 μg/ml. Table 1 shows the results obtained on the net flux of water as well as on the difference in electrical potential across the skin.

It can be seen that curare at the concentration used consistently enhanced the net flux of water. In some instances the flux rate was five times that of the control, although in most experiments the rate was enhanced two- to threefold. These results are consistent with the hypothesis that curare acts on the frog skin by increasing the diameter of membrane pores. However, it is important to note that, despite the apparent increase in membrane permeability to water, the membrane potential was not consistently altered.

The following conclusions may be drawn from these results. Although some of the results are contradictory to the proposed mechanism, it may be suggested that in these cases, for still obscure reasons, an intracellular increase of sodium concentration due to an increase in passive diffusion may not always enhance active transport. On the other hand, the hypothesis first proposed to explain the effect of curare on the active transport of sodium may not be correct, in that an increase in net flux of water and an increase in passive sodium permeability may result from two different mechanisms. Finally, the enhancement of active transport by curare cannot be explained in terms of an increase in the passive permeability to sodium (*5*).

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Table 1. Effect of curare on the potential difference and net flux of water across the isolated skin of *Rana temporaria temporaria* L. (Ringer's solution inside, Ringer at 1:10 outside). The time in hours indicates the duration of the control or experimental periods. Δ Potential difference is the maximum variation of the potential difference observed after application of 170 μg/ml of *d*-tubocurarine chloride in the outside solution. The minus sign indicates an increase in potential difference.

Experiment No.	Control		Curare		
	Time (hr)	Net flux of water ($\mu l\ cm^{-2}\ h^{-1}$)	Time (hr)	Net flux of water ($\mu l\ cm^{-2}\ h^{-1}$)	ΔPotential difference (mv)
1	4	5	4	8	–
2	4	3	3.5	7	4
3	4	3	4	4	11
4	4	3	3	16	6.5
5	4	3	3.5	15	7
6	3.5	3	3.5	9	4
7	3	5	1.5	12	−9.5
8	3	4	2.5	11	−1.5

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Excretion of Dopamine in Diseases of Basal Ganglia

Abstract. The urinary excretion of catecholamines has been measured in 32 patients with disorders of the basal ganglia. Sixteen patients with Parkinsonism (idiopathic, postencephalitic, and arteriosclerotic types) had a significantly lower amount of dopamine in the urine during a 24-hour period than a group of 24 normal control subjects. In a group of 16 patients with various striatal syndromes the excretion of dopamine and epinephrine was significantly higher than normal. Norepinephrine excretion was similar in the three groups. The lowest mean value of urinary dopamine was found in postencephalitic Parkinsonism; the highest occurred in Wilson's disease.

Recent chemical studies have revealed that 80 percent of the dopamine (3-hydroxytyramine) content of the brain is located within the corpus striatum (*1*). The differential concentration of norepinephrine and dopamine in