Table 1. Effects of magnesium pemoline (MgPe), $5 \times 10^{-8}M$ in vitro on brain RNA polymerase. The incubation medium was the same as in Fig. 1 except that CTP-H^3 (specific activity, 100 $\mu c/\mu$mole) was used instead of GTP-α-P^{32}. The enzyme was aged for 24 hours at −25°C prior to assay. The activity is expressed as picomoles of CMP incorporated into RNA per milligram of protein in 12 minutes. Values are mean ± standard deviations.

Additions	Polymerase		4-NT/1-NT
	True RNA (4-NT)	Pseudo-RNA (1-NT)	
None	7.4 ± 0.9	1.2 ± 0.1	6.2
MgPe*	7.2 ± 0.9	1.1 ± 0.1	6.5
DMSO, 0.02*M*	9.1 ± 1.0	1.2 ± 0.1	7.6
MgPe in 0.02*M* DMSO	19.0 ± 2.1	0.9 ± 0.2	21.1

*Aqueous suspension.

phosphates (the true RNA polymerase reaction) is observed. In itself DMSO has little effect on either reaction system. While the data reported here were obtained with tritiated cytidine triphosphate as the labeled precursor, experiments with other ribonucleoside triphosphates as the labeled substrate yielded similar results. Magnesium pemoline also produced enhancement of the true RNA polymerase activity (*5*) when enzyme was prepared by other methods of isolation (*6*).

Magnesium pemoline is pharmacologically classed as a mild stimulant of the central nervous system. The ratio of true to pseudo-RNA polymerase was determined for other psychotropic agents such as imipramine, methamphetamine, methylphenidate, pargyline, pipradol, and trimethadione and was found to be 0.60, 0.59, 0.54, 0.70, 0.67, and 1.0 respectively. These agents did not produce the selective activation of the true RNA polymerase system that magnesium pemoline did (ratio 1.95). While these data were derived from assays with the fresh enzyme preparation, relations were similar when an aged enzyme preparation was used. Thus the effect of magnesium pemoline is specific and not necessarily related to the general pharmacological properties of psychotropic drugs. Moreover, the differential activity of these agents on the two types of activities further strengthens the hypothesis that two separate and distinct enzymic activities are present in the nuclear aggregate.

The mechanism by which magnesium pemoline activates the nuclear aggregate enzymes responsible for RNA synthesis cannot be definitively determined from our data. Among the possible explanations of the observations could be (i) direct activation of the enzyme or enzymes, or (ii) an allosteric alteration of a single enzyme protein molecule, or (iii) an activation of the DNA primer to make it a more effective template.

Plotnikoff (*7*) has reported that magnesium pemoline enhanced acquisition rate and retention of conditioned avoidance performance in rats. While a causal relationship between learning and RNA synthesis cannot be deduced from our data, agents such as magnesium pemoline might provide a means to establish this relationship.

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Magnesium Pemoline: Enhancement of Learning and Memory of a Conditioned Avoidance Response

Abstract. *Magnesium pemoline, a mild stimulant of the central nervous system, enhances the acquisition and retention of a conditioned avoidance response in rats. Methamphetamine and methylphenidate do not have this effect.*

There have been several reports suggesting that changes in the RNA and DNA content of the brain may be directly related to processes of learning and memory (*1*). Daily RNA injections in rats increase acquisition and retention of a conditioned avoidance response (*2*). In presenile patients treated with RNA memory improved (*3*). Chamberlain, Rothschild, and Gerard (*4*) reported that tricyanoaminopropene stimulated nucleic acid levels and acquisition rates of conditioned avoidance responses.

Glasky and Simon (*5*) have reported that magnesium pemoline stimulates RNA polymerase in rat brain and predicted that magnesium pemoline should have an effect on learning and memory. Magnesium pemoline (*6*) is a stimulant acting on the central nervous system but devoid of sympathomimetic activity (*7*). The effects of magnesium pemoline on the acquisition and retention of a conditioned avoidance response in rats are now reported.

Male Sprague-Dawley rats (170 to 220 g) were used. The rats were maintained in a stock colony (six rats per cage) and handled only during the time of testing. The testing equipment (*8*) consisted of a wood chamber with a grid flooring and an escape platform outside of the chamber placed 30 cm

Table 1. The effect of magnesium pemoline on the acquisition phase of the jump-out response. There were six rats for each dose. Results represent the mean (in seconds) of the record "jump-out" time (mean ± S.E.).

Dose			Saline controls
5 mg/kg	10 mg/kg	20 mg/kg	
26.7±0.3	26.5±0.2	25.7±1.3	26.5±0.9
14.7±2.7*	14.5±3.0	20.0±2.2	27.7±1.4
10.5±2.3	8.8±2.0	9.7±3.2	26.5±2.1
11.0±3.4	8.3±2.0	5.8±0.8	22.3±2.5
8.2±1.0	9.0±2.4	5.7±1.1	21.0±3.8
6.0±0.6	6.7±1.2	4.5±0.4	21.2±2.6
6.7± .9	6.2±0.9	4.0± .9	15.0±3.2
8.5±1.8	4.2± .5	4.0± .8	14.0±3.4
5.0±0.7	4.3± .8	3.5± .7	14.8±2.8
5.2± .9	4.3± .7	3.5± .6	16.7±2.0

*Statistics (*13*): In trials Nos. 2–10, groups at all doses had mean jump-out times significantly different from controls ($P < .05$). Between trials Nos. 1 and 10 there was a significant linear decrease in mean jump-out times from trials 1 to 10 ($P < .05$) in all groups.

above the grid floor. The electric shock to the grid floor was controlled by a rheostat mechanism and scrambler. On the 1st day of the experiment, the rats were given a series of three trials with the following 30-second sequence: 15 seconds inside the chamber without shock or buzzer, 10 seconds with buzzer, and finally 5 seconds of shock with buzzer. The three test trials on day 1 were used to select "slow learners" for all subsequent studies of the drug. Slow learners were those that escaped only to applied shock during the last 5 seconds of the 30-second sequence. On the 2nd day, the test rats were given the drug orally (5 to 20 mg) 30 minutes prior to the first acquisition trial of a ten-trial sequence. On the 3rd day, retention of the jump-out response was measured by placing the test rat inside the chamber for 30 seconds without any buzzer or shock stimulation. The time from entrance into the apparatus until the rat jumped out onto the wire screen leading onto the platform was recorded as the jump-out time in seconds. The criterion of learning in this test was a mean jump-out time of 15 seconds.

Table 2. Effect of magnesium pemoline on the retention phase of the jump-out response. Results are expressed as the mean jump-out time in seconds ± S.E., six rats at each dose, ten trials. All trial and treatment means differ significantly from control means ($P \leq .05$). There was a significant linear increase from trial 1 to trial 10 for the control group ($P \leq .05$), while there was no significant difference among the mean jump-out times of the ten trials for each of the three drug-treated groups.

Dose			Controls
5 mg/kg	10 mg/kg	20 mg/kg	
5.5±0.6	5.7±1.0	5.7±1.1	15.3±2.9
5.8± .3	5.3±0.8	5.0±0.6	13.2±1.7
5.3± .5	4.5± .6	3.7± .6	14.0±2.5
6.3± .5	4.8± .4	4.0± .8	13.3±1.7
6.3± .6	5.8± .5	3.3± .8	14.7±1.7
6.5± .7	4.3± .5	4.5± .7	16.2±3.1
6.2± .7	5.8± .7	5.3± .9	19.3±2.8
6.5± .6	5.7± .5	5.5±1.2	21.3±2.7
7.2± .9	6.5± .9	4.2±0.7	22.2±3.7
5.7± .6	5.7± .9	5.0± .7	23.0±3.1

Table 3. Effect of methylphenidate and methamphetamine on the acquisition and retention of the jump-out response. Results are given as the mean jump-out time in seconds ± S.E., ten trials in each phase.

Intraperitoneal dose		Saline controls
Methylphenidate (5 mg/kg)	Methamphetamine (1 mg/kg)	
Acquisition phase		
27.3 ± 0.6	26.8 ± 0.4	27.5 ± 0.8
25.8 ± 1.9	20.5 ± 3.4	27.2 ± 0.9
20.0 ± 2.7	20.5 ± 2.7	24.2 ± 2.5
18.3 ± 3.0	18.7 ± 3.2	22.8 ± 2.8
17.8 ± 2.4	17.8 ± 2.1	19.3 ± 2.8
13.3 ± 2.8	13.7 ± 1.4	11.8 ± 3.3
11.5 ± 2.4	13.8 ± 1.8	11.0 ± 1.8
10.0 ± 1.9	15.5 ± 2.7	10.0 ± 3.1
12.8 ± 3.0	16.3 ± 1.8	10.0 ± 3.3
11.8 ± 3.1	17.3 ± 2.1	8.5 ± 2.8
Retention phase		
25.0 ± 2.3	28.2 ± 1.4	24.5 ± 1.6
27.0 ± 1.4	27.2 ± 2.0	24.8 ± 1.7
28.0 ± 1.8	29.6 ± 0.2	25.8 ± 1.8
28.2 ± 1.2	28.4 ± 1.2	25.5 ± 1.7
28.3 ± 1.7	27.8 ± 2.2	28.2 ± 0.8
29.5 ± 0.5	27.6 ± 2.4	28.2 ± 1.1
30.0 ± 0.0	28.0 ± 2.0	28.2 ± 1.5
30.0 ± 0.0	22.2 ± 5.1	27.0 ± 2.1
30.0 ± 0.0	27.8 ± 2.2	26.8 ± 2.0
30.0 ± 0.0	25.8 ± 2.6	25.8 ± 2.7

Twenty-four hours after the acquisition trials the jump-out response of the drug-treated groups was retained for a long period (Table 2). The drug-treated groups uniformly escaped within 3 to 8 seconds after placement in the test chamber. Controls failed to maintain their previously learned escape response on the retention trials and rapidly showed a decline in performance (from 13 to 23 seconds average escape time over a period of ten retention trials) (*9*).

In sharp contrast to magnesium pemoline, both methamphetamine (0.1 to 2 mg/kg) and methylphenidate (2.5 to 20 mg/kg) were completely ineffective in altering acquisition or retention responses (Table 3). No significant differences in acquisition rates between the three groups were observed. Methylphenidate and methamphetamine did not alter retention responses as compared to controls.

Thus magnesium pemoline enhances the acquisition and retention of a conditioned avoidance response in rats. This enhancement by magnesium pemoline of learning and memory in the "jump-out" test is unusual since both methamphetamine and methylphenidate are inactive and since it was observed at non-stimulant doses (no increases in spontaneous motor activity). Similar ineffectiveness for amphetamine has been reported by Bovet (*10*) in a shuttle-box test and by Maffii (*11*) in a pole-climbing test. These negative findings with amphetamine in the gross operant avoidance test systems (jump-out, pole climb, hurdle cross) are contrasted sharply to positive effects in facilitating acquisition in the instrumental (bar-pressing) avoidance test (*12*). Certainly, differences of such magnitude between the effects of amphetamine and magnesium pemoline on acquisition rates in the gross operant jump-out test may be explained in terms of possible changes in anxiety or alertness levels or both.

Whether or not enhancement of learning and memory by magnesium pemoline in rats is causally related to the biochemical effects of magnesium pemoline (*4*) cannot be definitively established from these studies. However, magnesium pemoline should provide a useful new tool in studying the biological basis of learning and memory.

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