upon the logical premise that the tracer cores were eroded from the top down and that the sample cores were deposited from the bottom up.

The efficacy of this procedure in integrating the depth parameter into fluorescent tracer techniques appears to be supported by my experiments. Further refinement of field and analytical methods should make the procedure a valuable tool adaptable for investigations of a wide variety of regimes.

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Magnesium Pemoline: Enhancement of Brain RNA Polymerases

Abstract. *The stimulation by magnesium pemoline of systems that synthesize brain nucleic acid was studied in vivo and in vitro. There are differential effects between true RNA polymerase and pseudo-RNA polymerase. The selective stimulation of true RNA polymerase by magnesium pemoline was not observed with stimulants of the central nervous system and psychotropic agents.*

Many investigators (*1*) have attempted to establish a functional relation between nucleic acid or protein metabolism (or both) and various aspects of brain function. Development of an agent which stimulates synthesis of brain nucleic acid and enhances learning would further strengthen the hypothesis that nucleic acids function as the informational engram in the brain. Magnesium pemoline (*2*), a stimulant of the central nervous system, enhances brain nucleic acid synthesis both in vivo and in vitro.

We have prepared a nuclear aggregate by the method of Barondes (*3*) and assayed it for ability to incorporate a radioactively labeled ribonucleotide into RNA (*4*). The activity was measured both in the presence of all four nucleoside triphosphates (4-NT reaction, true RNA polymerase) and in the presence of only one nucleoside triphosphate (1-NT, pseudo-RNA polymerase).

Magnesium pemoline was administered intraperitoneally (20 mg/kg) to Sprague-Dawley white rats (250 to 300 g). Groups of 6 to 10 animals were killed at intervals after treatment with magnesium pemoline, and the nuclear aggregate was prepared from the pooled brain tissue of these animals. The control group did not receive the drug, and the brain tissue was collected and processed simultaneously with the brain tissue from the treated groups. The nuclear aggregate preparation derived from each group was assayed for true and pseudo-RNA polymerase activities (Fig. 1).

While the pseudo-RNA polymerase activity showed a more rapid rate of increase initially, the activity reached a maximum and remained constant after approximately 30 minutes. The true RNA polymerase activity was lower than the pseudo-RNA polymerase activity at first but showed a linear and continuous increase in activity as a function of time. The value for the ratio of the activity of true to pseudo-RNA polymerase exceeded 1.0 at about 1 hour and remained greater than 1.0 thereafter. One of the possible explanations for the kinetics observed under our conditions is that there are two different enzymatic activities in the nuclear aggregate preparation and that each is activated by magnesium pemoline at a different rate.

Since magnesium pemoline caused activation in vivo of the RNA synthetic enzymes, we studied the effect of magnesium pemoline added in vitro on the activity of a nuclear aggregate enzyme prepared from the brain tissue of nontreated animals (Table 1). When the enzyme preparation had been aged for 24 hours at −25°C prior to assay, there was some enhancement of the enzyme activities and particularly an enhancement of the true RNA polymerase activity. The ratio of true to pseudo-RNA polymerase was approximately 6, as opposed to 1.0 or slightly less with a fresh preparation. The results with our aged enzyme preparation are in agreement with those reported by Barondes (*3*). The lack of any effect by the aqueous suspension of magnesium pemoline on the aged enzyme preparation was due to the extreme insolubility of the drug in water. Dimethyl sulfoxide partially solubilizes magnesium pemoline and once magnesium pemoline has been solubilized, a pronounced and preferential activation of the incorporation of ribonucleotides into RNA in the presence of all four nucleoside tri-

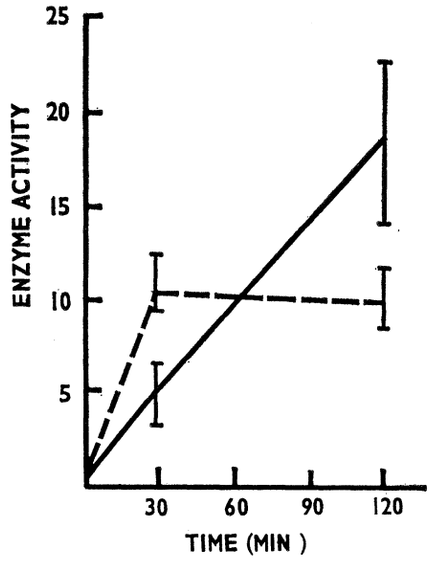

Fig. 1. Effect of magnesium pemoline (20 mg/kg, intraperitoneally) on activities of brain RNA polymerase in vivo. The 1-NT incubation mixture contained: tris buffer, *p*H 8.0, 100 μmole; $MgCl_2$, 10 μmole; KCl, 1 mmole; NaF, 40 μmole; cysteine, 10 μmole; phosphoenolpyruvate, 2 μmole; pyruvate kinase, 100 μg; GTP-α-P^{32} (specific activity, 100 μc/μmole), 0.1 μmole; enzyme, 1.0 mg protein in a final volume of 2.0 ml. The 4-NT incubation mixture was the same as the 1-NT incubation mixture except for the addition of 0.1 μmole each of ATP, CTP, and UTP. The mixture was incubated at 37°C for 12 minutes, the reaction was terminated by the addition of 0.1 ml of 4 percent sodium pyrophosphate and 5.0 ml of 10 percent trichloroacetic acid, and the incorporation was determined by the Millipore-filter method. The enzyme activity is expressed as picomoles of GMP incorporated into RNA per milligram of protein in 12 minutes. The vertical bars represent the range of enzyme activities observed under each experimental condition. Solid line, tris RNA polymerase; dotted line, pseudo-RNA polymerase.

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.