puter system. A vertical line was then positioned by a computer program at each inflection point of the displayed wave pattern; these lines then served as break points for analysis of data. The amplitude values of the raw data of the segment of the wave between two break points was then averaged and stored. By this device, the relative amplitude of comparable segments of different waves could be statistically compared by using Student's t test. This method allows some estimate of the reliability (in the face of variability) of the difference between segments of the waveform, though, of course, it does not determine whether a total waveform is significantly different from another. When reported, a waveform differs from its control at least by $P < .05$.

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Pemoline and Magnesium Hydroxide: Lack of Effect on RNA and Protein Synthesis

Abstract. *Brain RNA polymerase isolated from rats treated with pemoline and magnesium hydroxide (Cylert) was not more active than enzyme from control animals. The drug did not increase enzymic activity in vitro. Pemoline did not significantly affect either RNA or protein synthesis in suspensions of Ehrlich ascites carcinoma cells.*

In a recent publication, Glasky and Simon (*1*) described the stimulating effect of magnesium pemoline (*2*) upon brain RNA polymerase. Data were presented which purported to show that the enzyme isolated from rats treated with the drug was more active than that from untreated animals, and that the chemical enhanced RNA synthesis when added to the in vitro assay system.

Repeated attempts by us to reproduce the original experimental observations have been unsuccessful. Typical experiments are described below (*3*).

The nuclear aggregate brain RNA polymerase as described by Barondes (*4*) was prepared from four groups of Sprague-Dawley white rats (100 to 150 g) which had received intraperitoneal injections of 20 mg of pemoline and magnesium hydroxide per kilogram of body weight in 0.25 percent Methocel, and which were then killed at varying times after the treatment. The brains from each of the groups were pooled for enzyme assay. The data obtained (Fig. 1) showed that (i) pemoline and magnesium hydroxide in vivo did not significantly affect brain RNA polymerase activity in any of the groups, (ii) there was significant enzyme activity at zero time, that is, in the group which was killed at the time of injection, and (iii) at no time was the enzyme more efficient in polymerizing a single triphosphate (1-NT reaction) than a mixture of all four triphosphates (4-NT reaction). Two other experiments with CTP-^{3}H confirmed these observations.

These findings are in contrast to the results obtained by Glasky and Simon in a similar experiment (*1*, Fig. 1). In addition to a positive drug effect, they reported almost no enzymatic activity without drug administration, and a 1-NT activity larger than 4-NT in their 30-minute groups. The latter two points also do not agree with the original observations on the rat-brain aggregate enzyme described by Barondes (*4*).

While this paper was in preparation, Morris *et al.* (*5*) reported that intraperitoneal administration of pemoline and magnesium hydroxide did not significantly affect either the concentration of rat-brain RNA or the uptake of tritiated uridine into brain RNA. We draw the same conclusions from similar experiments carried out in this laboratory.

Results obtained in 16 experiments in which drug was added directly to the in vitro assay system also failed to substantiate the original report. Table 1 shows that neither pemoline nor pemoline and magnesium hydroxide exerted a significant effect upon the polymerase activity.

Although it was not expected that pemoline and magnesium hydroxide would yield different results from that of pemoline alone in an in vitro system optimized with respect to divalent cation requirement, the former was included for comparison with the previous report (*1*). Most of our experiments were carried out with aqueous solutions of the drugs. Since the solubility of pemoline in water is approximately 0.2 mg/ml ($> 10^{-3}$ mole/liter) we felt that the use of dimethylsulfoxide, as suggested in the original report, was unnecessary (*1*). Nevertheless, some of our experiments did include pemoline and magnesium hydroxide "solubilized" in dimethylsulfoxide, but again no drug effect was observed.

Pemoline and magnesium hydroxide (1 and 5×10^{-5} mole/liter) did not increase the incorporation of CTP-α-^{32}P into RNA of isolated rat-brain nuclei in vitro.

While brain has a high rate of nucleic acid and protein synthesis, we

Table 1. Lack of effect of pemoline or pemoline plus magnesium hydroxide on brain RNA polymerase. The assay system in vitro was the same as in Fig. 1, except that the following ribonucleoside triphosphates were used per 2.0 ml: ATP, GTP, UTP, 0.9 μmole each; CTP-^{3}H, 100 μc/μmole, 0.02 μmole. Enzyme activity is expressed as picomoles CMP incorporated per milligram of protein in 15 minutes; mean values (triplicate) ± standard deviation are cited.

Addition (mole/liter)		Enzyme activity
Pemoline	Pemoline + $Mg(OH)_2$	
Reaction stopped at zero time		4 ± 0.4
0	0	43 ± 6
1×10^{-5}	0	41 ± 3
5×10^{-5}	0	37 ± 5
1×10^{-4}	0	39 ± 7
0	1×10^{-5}	46 ± 8
0	5×10^{-5}	44 ± 3
0	1×10^{-4}	38 ± 2

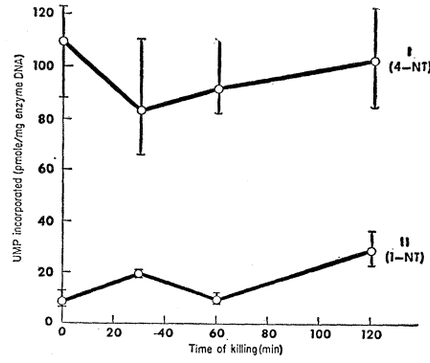

Fig. 1. RNA polymerase activity from rat brain of four groups, each containing ten rats. All the animals received 20 mg of pemoline and magnesium hydroxide per kilogram of body weight and were killed at the times shown after drug administration. The enzyme activity assay system for curve I (4-NT) contained per 2.0 ml: Tris buffer, pH 8.0, 200 μmole; $MnCl_2$, 4 μmole; KCl, 1000 μmole; ATP, CTP, GTP, 0.5 μmole each; UTP-α-^{32}P, 150 μc/μmole, 0.02 μmole. For curve II (1-NT), ATP, CTP, and GTP were omitted. The DNA content per 2.0 ml was 0.175, 0.176, 0.192, and 0.176 mg for the 0-, 30-, 60-, and 120-minute enzyme preparations, respectively. The mixtures were incubated for 15 minutes at 37°C; at this time, 0.1 ml of a 4-percent $Na_4P_2O_7$ and 0.5-percent RNA solution was added, and the reaction was terminated by the addition of 5 ml of 10 percent trichloroacetic acid. The precipitates were washed three times with cold 5-percent trichloroacetic acid on Millipore filters, and the radioactivity was determined in a liquid-scintillation counter. The activity is expressed as the number of picomoles of UMP incorporated per milligram of DNA in 15 minutes (1 picomole is equivalent to 200 count/min; the zero time control, 327 count/min). The vertical bars represent the range of activities, determined in triplicate. If $MnCl_2$ was eliminated from the incubation medium, the activity was reduced to 5, slightly above background.

have no reason to believe that macromolecular synthesis in nervous tissue is qualitatively different from that in any other tissue. Therefore, an agent which directly affects these processes in brain cells might reasonably be expected to do so in other cell types in vitro. Accordingly, we tested for possible effects of pemoline on RNA and protein synthesis in suspensions of Ehrlich ascites carcinoma (EAC) cells. RNA synthesis was followed by the incorporation of tritiated uridine (U-5-^{3}H) into material insoluble in trichloroacetic acid (TCA) and a mixture of ethanol and ether. The cells (approximately 10^6 per milliliter) were suspended in Eagle's minimal essential medium (spinner modified) and incubated in air at 37°C. After 10 minutes, 0.5 μc of U-5-^{3}H (4 c/mmole) was added, and the incubation was continued for 30 minutes. The reaction was stopped by the addition of 4 ml of ice-cold, 5-percent TCA, and the resulting precipitate was washed twice in TCA. Lipids were extracted once with ethanol and twice with a mixture of ethanol and ether (3 : 1). The residue was dried in a hot-water bath, dissolved in formic acid, and transferred to counting solution; the radioactivity was then measured in a liquid-scintillation counter. Similarly, protein synthesis was determined with the use of ^{14}C-L-valine (0.5 μc; 160 mc/mmole). In these experiments the cells were suspended in spinner salt solution, and a TCA wash (15 minutes; 90°C) was added to the work-up. During the 30 minute incubation period pemoline did not significantly affect RNA or protein synthesis in EAC cells (Table 2). Similar results were obtained in eight separate experiments.

Additional experiments on protein synthesis were carried out in a respiration-dependent protein-synthesizing system of rat-brain mitochondria similar to that described by Campbell *et al.* (*6*). Pemoline in concentrations up to 5×10^{-4} mole/liter did not affect the incorporation of ^{14}C-L-leucine into the fraction insoluble in hot TCA. From this we tentatively conclude that the stimulant activity of the drug on the central nervous system is not due to an action on respiration or oxidative phosphorylation.

Since the results reported by Glasky and Simon (*1*) could conceivably be due to an indirect effect of pemoline on adventitious ribonuclease, we tested the drug in the standard Kunitz assay for ribonuclease activity (*7*). We used crystalline bovine pancreatic ribonuclease and yeast RNA. Pemoline (5×10^{-4} mole/liter) did not inhibit the enzyme.

Table 2. Lack of effect of pemoline on RNA and protein synthesis in Ehrlich ascites carcinoma cells. Data are given for duplicate samples.

Addition	Incubation time (min)	Count/min
Uridine precursor		
None	0	44
		40
None	30	14,845
		14,435
Pemoline ($2.5 \times 10^{-4}M$)	30	14,195
		13,805
Actinomycin D (5 μg/ml)	30	345
		392
Valine precursor		
None	0	25
		26
None	30	4,008
		3,663
Pemoline ($2.5 \times 10^{-4}M$)	30	3,876
		3,283
Cycloheximide (7 μg/ml)	30	1,715
		1,562

At the present time we have no biochemical explanation to offer for the behavioral effects described by Plotnikoff (*8*). However, in view of the results reported here and those of Morris *et al.* (*5*), we feel that the implications raised by Glasky and Simon (*1*) are not justified.

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References and Notes

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2. Pemoline and magnesium hydroxide (Cylert, Abbott-30400): a combination of 2-imino-5-phenyl-4-oxazolidinone and magnesium hydroxide.
3. The following abbreviations are used: ATP, CTP, GTP, and UTP for the ribonucleoside triphosphates of adenine, cytosine, guanine, and uracil, respectively; CMP and GMP for the ribonucleoside monophosphates of cytosine and guanine, respectively; and UMP for uridine monophosphate.
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Animals at Very High Pressures of Helium and Neon

Abstract. *At pressures up to 125 atmospheres, helium failed to anesthetize mice; at slightly higher pressures (135 to 145 atmospheres) it proved lethal. With Italian newts* (Triturus italicus), *whose sensitivity to anesthesia by nitrogen is similar to that of mice, responsiveness was lost at pressures between 165 and 245 atmospheres, whether the pressure was achieved with helium or neon, or hydrostatically. It was concluded that the anesthetic pressures of helium and neon, for mice and newts, are higher than the tolerable mechanical pressures.*

While the effects of very high pressures (of the order of 100 atm or above) on isolated tissues and on vegetable materials have been studied extensively (*1*, *2*), experiments with living animals have been fewer. Carpenter (*3*), by extrapolation according to the dose-response relation observed with other gases, estimated the ED_{50} (dose that was 50 percent effective) for helium anesthesia to be > 163 atm. His maximum pressure was, however, very considerably below this. Membery and Link (*4*) reached a similar conclusion up to their maximum working pressure of 90 atm. Recent experiments with monkeys showed that at a pressure of 67 atm for a mixture of oxygen and helium severe convulsions occurred, as indicated by electroencephalographic recordings, whereas no such effects were produced by a mixture of oxygen and hydrogen under similar conditions (*5*).

In this work we report some preliminary observations made at high pressures. Male white mice (C. D. Tuck No. 1 uniform strain) and Italian great newts (*Triturus italicus*) have been studied at pressures up to 245 atm. A 300-ml stainless steel pressure vessel fitted with a perspex port was used. In all experiments the vessel was flushed with oxygen before the inert gas was added under pressure, and strict precautions were taken to control the carbon dioxide partial pressure. The environmental temperature was controlled by placing the chamber in a water bath. Anesthetic end-points were determined by the rolling-response (RR) method, based on the ability of the animal to remain upright when the chamber was rotated. A more detailed description of the experimental technique will be given elsewhere (*6*).