sens might be employed to determine the distribution in size and concentration of tobacco smoke particles and droplets before and after inhalation and so determine the percentage of droplets of each size range trapped in the respiratory system. Thus the method of studying, by high-power optical microscopy, tobacco smoke particles supported on invisible spider threads may well be a useful addition to techniques currently in use.

Aside from their use in the capture of objects of submicron diameter, the invisible threads make it possible to perform a variety of experiments on single microscopic particles. For example, scattering experiments can be performed on a thread-supported microscopic water drop in the visible region of the spectrum without the necessity of employing levitation apparatus to support the droplet. Evidently the only scattering experiments on a single microscopic droplet are those of Egan (*5*) and Rowell (*6*), who studied the scattering of visible radiation from a microscopic oil droplet suspended by means of a Millikan oil drop apparatus. On the other hand, we have found that scattering experiments can be performed on a microscopic water droplet formed by controlling the relative humidity of the environment of a small salt crystal caught on a submicroscopic spider thread (*7*). Because the diameter of the droplet is much larger than that of the invisible supporting thread, surface tension does not cause an appreciable deformation of the spherical droplet. Further, scattering theory predicts that the radiation scattered by the invisible thread will be orders of magnitude below that scattered by, say, a droplet 1 μ in diameter. Consequently, the supporting thread will probably affect neither the shape of the droplet nor the measurement of the scattered radiation.

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Magnesium Pemoline: Failure To Affect in vivo Synthesis of Brain RNA

Abstract. *The effect of magnesium pemoline on the synthesis of brain RNA in vivo was studied. No significant effect either on the concentration of RNA or on the uptake of H^3-uridine into RNA was detected.*

There has been much interest in drugs that influence brain RNA and protein synthesis, with respect to their effects on learning and memory (*1*). Recently, Glasky and Simon reported (*2*) that magnesium pemoline (Abbott 30400; Cylert, a combination of 2-imino-5-phenyl-4-oxazolidionone and magnesium hydroxide) enhanced the activity of the RNA polymerase of brain when the drug was studied under either in vivo or in vitro conditions. A concurrent report by Plotnikoff (*3*) indicated that the drug enhanced both learning and memory in a conditioned avoidance-response situation. Although a causal relation between stimulation of RNA synthesis and an effect on learning and memory was specifically disavowed, on the basis of the data reported, it was suggested that magnesium pemoline might be used to establish such a relation (*2*).

According to Glasky and Simon, at 30 minutes and 120 minutes after the intraperitoneal injection of magnesium pemoline (20 mg/kg) the "true" RNA polymerase activity of nuclear aggregates prepared from brains of treated animals was increased. A similar stimulation of enzyme activity was reported when the drug was added in vitro to RNA polymerase preparation, but only after the drug was made soluble with dimethyl sulfoxide. No attempt was made to measure directly the effect of the drug on synthesis of RNA in the brain.

If the reported effects on the activity of brain RNA polymerase are meaningful, an effect of the drug on synthesis of RNA in brain in vivo ought to be readily demonstrable. We have measured the effect of pemoline on the concentration of RNA in the brain and also on the amount of incorporation, into brain RNA, of injected H^3-uridine. The test rats (100 to 150 g, Sprague-Dawley strain) were given magnesium pemoline intraperitoneally in doses of 5, 10, 20, or 40 mg/kg (*4*). This range of doses brackets the dose previously used, 20 mg/kg (*2*). Animals in the control group were injected with water, or with $MgCl_2$ at the same concentration as pemoline; $MgCl_2$ had no apparent effect. One hour later, H^3-uridine (0.5 ml aqueous solution; 2.5 μmole, 100 μc/μmole) was injected into the tail veins of the rats. One hour after this injection (2 hours after the drug) the rats were guillotined; the brains were removed in the cold, and the RNA content and the specific activities were determined. Weighed samples of the tissues (0.3 to 0.5 g, one cerebral hemisphere) were homogenized briefly in 4 ml of ice-cold water to which was added 6 ml of 10 percent trichloroacetic acid (TCA) as soon after homogenization as possible. The precipitate was recovered by centrifugation and was washed six times with cold 5 percent TCA. After the last wash no radioactivity remained in the supernatant fraction. The final precipitate was drained well, 2 ml of 1*M* NaOH was added, and the samples were incubated for 16 hours at 37°C. These samples were then acidified to precipitate DNA and protein, and the supernatant fraction was assayed for orcinol-reactive material and material absorbing at 260 mμ, as well as for radioactivity. The values obtained from the two methods of measuring the concentration of RNA-nucleotide were

Table 1. Effect of pemoline on the concentration of RNA in the brain and the specific activity of RNA in both control and pemoline-treated rats. Each value represents the mean value for four animals ± the standard error of the mean. When experimental mean values are compared to control mean values for each experiment, the differences are not statistically significant according to Student's *t*-test.

Pemoline (mg/kg)	Experiment	Brain RNA (μg/g)		Specific activity of RNA (count/min per microgram)	
		Control	Pemoline-treated	Control	Pemoline-treated
5	A	1654 ± 76	1705 ± 103	1.19 ± 0.09	0.98 ± 0.22
10	A	1654 ± 76	1578 ± 154	1.19 ± .09	1.16 ± .28
10	B	1601 ± 63	1584 ± 51	1.29 ± .10	1.50 ± .17
20	C	1684 ± 83	1660 ± 23	1.98 ± .20	1.70 ± .15
20	D	1752 ± 32	1515 ± 70	1.42 ± .07	1.38 ± .11
40	B	1601 ± 63	1561 ± 66	1.29 ± .10	1.22 ± .10

within 10 percent of one another. The content of RNA in the tissues and the specific activity of the RNA were then calculated. We were unable to find any statistically significant increase of either the RNA content of the brain or the incorporation in vivo of H^3-uridine into brain RNA (Table 1) in response to administration of pemoline. In fact, the pemoline-treated animals may show a slight decrease in RNA content (*5*).

In order to reconcile the seeming discrepancy between our results and those reported by others (*2*), it might be argued that the effect of the drug is to stimulate the synthesis not of the total RNA of the brain, but of a special (rare) class of RNA. It might be supposed that only this special class of RNA is synthesized by the RNA polymerase that was measured in vitro by Glasky and Simon (*2*), and that the RNA polymerase responsible for bulk synthesis of RNA is undetectable under these same conditions in vitro. According to this argument, pemoline would have an effect in vitro but not in vivo, but there is no compelling reason to believe that this may be true. If there is no such special class of RNA, another argument to explain the fact that total brain RNA does not increase in response to pemoline might be that the drug increases the turnover of brain RNA, so that, although there is increased synthesis of RNA, it is balanced by rapid breakdown. If this were so, however, one would expect that the RNA from the drug-treated animals would have substantially higher specific activities than the controls. This was not observed. Although it is difficult to draw firm conclusions on the basis of negative findings, the data presented in this report suggest that much more work needs to be done before magnesium pemoline can be regarded as a drug that stimulates the synthesis in vivo of RNA in the brain.

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4. We thank R. K. Richards of Abbott Laboratories for providing us (through N. J. Giarman of the Department of Pharmacology) with samples of magnesium pemoline.
5. When all control and all treated animals were grouped and compared as two larger samples by Student's *t*-test, the value of P for the significance of the apparent decrease in total brain RNA for the pemoline-treated group was $0.1 < P < 0.5$. There was no statistically significant difference for the RNA specific activity measurements between the two combined samples.
6. We are grateful to Donna Reno for technical assistance. Supported by American Cancer Society grant P333 and PHS grant 5-T1-CA5055.

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Leukemia Mortality: Downturn Rates in the United States

Abstract. *A decline, the first ever observed, has recently occurred in leukemia mortality rates for the white population of the United States between the ages of 1 and 74. Possible explanations include diminished exposure to medical x-rays following the release in the United States and Great Britain in 1956 of widely publicized reports on the biologic effects of ionizing radiation.*

A progressive rise in leukemia mortality rates over time has been described in the United States (*1*), England (*2, 3*), and elsewhere (*4*). Among possible reasons for the increase are better diagnosis and reporting, improved survival from infections in the preleukemic phase, and increasing exposure to environmental leukemogens. To determine the extent to which these influences are still operative, we have examined recent leukemia trends in the United States and in England and Wales.

To U.S. leukemia-mortality rates for 1921–60 (*1*) we added data through 1965 (*5*); Fig. 1A illustrates the trends for the white population, on semilogarithmic scale to enable visualization of the rate of change with the passage of time. In the most recent 5-year period, 1961–65, the leukemia rates were for the first time lower than those for the previous period in all age groups from 1 through 74 years, the decline being greatest among children aged from 1 to 4. The downturn was preceded by a variable interval of decelerated climb, such as would occur if improvements in case-detection were approaching their limits while other factors changed little. The earlier fall in leukemia mortality among children under 1 year has been attributed to reduction in wrong diagnoses, which are much more common among infants than for older patients (*6*).

The trends for U.S. nonwhites between 1921 and 1965 (Fig. 1B) were less regular because of the small number of deaths in each category considered. Generally, the rates for the last decade tended to level off, but there was no consistent recent downturn as shown by the white population.

To determine whether the decrease noted for U.S. whites also occurred elsewhere, we examined statistics for England and Wales from 1921 to 1964 (*7*); in availability of published data and in characteristics of medical practice and population, this area resembles the United States. Because deaths from leukemia were fewer in England and Wales, the rates were more variable than among U.S. whites. While the trends for the two populations were similar until 1960, a decline in rate during the last time-period in England and Wales occurred only among children under 5 years. The rates for persons from 5 to 49 years have leveled off, but for the older age groups a slight rise has continued.

If the long-term increase in mortality from leukemia was partially the result of environmental factors, the recent decline among the U.S. white population suggests that these factors have become less prevalent or less effective with time. Since the rates for U.S. nonwhites and for England and Wales are still in the process of stabilizing, it appears that any decrease in environmental factors was greater, or occured earlier, among U.S. whites. In explaining the racial differences in the United States one should consider the probability that, in the past, diagnosis and reporting of leukemia were more complete among whites than nonwhites, so that the recent trends among nonwhites may reflect a narrowing of this gap.

It is of interest that the largest reduction in rates among U.S. whites, and the only decline in rates for England and Wales, occurred in children under 5 years. Although this phenomenon may have resulted from lengthening of the duration of survival of leukemia, with a shift in mortality to greater ages, certain laboratory (*8*) and epidemiological (*9*) observations suggest that the mortality experience of young children is the most sensitive indicator of environmental leukemogens.

While factors responsible for the downturn in mortality from leukemia among U.S. whites are unknown, one may suspect environmental exposures which have varied concomitantly with