out the 'S' phase the number of silver grains/mitosis should have increased for the duration of the 'S' phase or until the thymidine H^3 was no longer available. Since the number of silver grains increased from 1.5–3.5 h after treatment the 2 h period was taken as the availability time of thymidine and as advocated by KOBURG[7] the plateau of the curve, equal to 6.5 h, was taken as the duration of the 'S' phase.

A further method was employed to check the value of the 'S' stage. Knowing the generation time, 10.0–10.5 h, and the labeling index 65%, the following formula can be applied (QUASTLER and SHERMAN[8]):

$$\frac{NS}{NC} = \frac{TS}{TG} = 65 = \frac{TS}{10\text{–}10.5} = 6.5\text{–}6.8.$$

This 6.5–6.8 h value for the 'S' stage agrees reasonably well with the value arrived at by the grain count method.

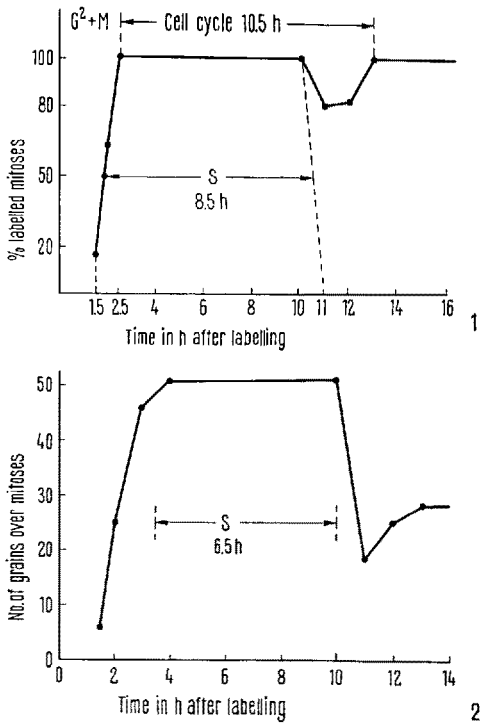

Fig. 1. Curve representing the percentage of labeled mitoses plotted against time between treatment with thymidine H^3 and sacrifice.

Fig. 2. Curve demonstrating the average number of silver grains per mitosis plotted against time between treatment with thymidine H^3 and sacrifice.

Discussion. As is apparent from the results, a discrepancy exists in the values for the 'S' stage with the 8.5 h value derived from the method of labeled mitoses being least compatable. This value was arrived at assuming that the thymidine H^3 was available for only a short period of time. As shown, however, the thymidine H^3 was available for at least 2 h during which time cells, initially outside the 'S' stage, could enter and incorporate the label thus lengthening the value of the synthesis stage. By utilizing the 2 h availability time we find an 'S' stage (8.5–2), equal to 6.5 h, a value which agrees with the time derived from the other 2 methods. Furthermore, if the values of G2 = 1.5 + M = 1.0 + S = 6.5 are now added together and the value of 9.0 h subtracted from the generation time of 10.0–10.5 h we have a value of 1.0–1.5 h for the G1 phase of the cell cycle.

In Figure 1 the descending curve does not fall below 80% labeled mitoses. A reasonable explanation may be as follows: the number of cells in a particular stage of the cell cycle is proportional to the time spent in that stage, therefore approximately 65% of all the neural tube cells are in the 'S' stage at any one time. Of the 35% unlabeled cells, approximately half will enter the 'S' stage and incorporate the label during the 2 h availability time of thymidine H^3. This means that at least 80% of all the cells within the neural tube will be labelled and, since there is a non-synchronous movement of cells through the cycle, as evidenced by the slope of the ascending and descending curves, the number of labeled cells should never drop below this 80% mark.

Résumé. Des études autoradiographiques effectuées sur les stades du cycle cellulaire dans les cellules neuroépithéliales de poulet de 2 jours, ont donné les résultats suivants: G2 = 1,5 h; M = 1,0 h; S = 6,5–6,8 h; G1 = 1,0–1,5 h et le temps de génération = 10,0–10,8 h. On a également constaté que la thymidine H^3 administrée à l'embryon comme dans l'expérience ci-dessus mit au moins 2 h à être incorporée.

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Short-Term and Long-Term Radioprotective Effect of Magnesium Pemoline

Radioprotective effect of magnesium pemoline was recently reported[1]. Further investigations showed that this drug is more potent than pemoline alone (made up 75% weight of the drug) in exhibiting protection against ionizing radiations. Results from the above studies have strongly indicated that magnesium pemoline offers significant degree of protection against lethal doses of X-irradiation for both short-term and long-term. In subsequent experiments we have studied explicitly this aspect and results are presented in this paper.

Methods. Two experiments were performed. In the first experiment, 540 CF-1 male mice (20–22 g), 50–60 days old were randomly divided into 3 groups. On the first day of the experiment half of the mice in each group were injected i.p. with 0.6 cm^3 of magnesium pemoline prepared in

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0.3% tragacanth suspension, and the other half with bacteriostatic water (0.3% of tragacanth). The drug dose for each mouse was approximately 70 mg/kg. 5–10 min after injection, one group of mice (Group I) was exposed whole-body to 750 R of X-irradiation. The irradiation techniques were described throughoutly in [1]. One week and 2 weeks after injection respectively, group II and group III were then exposed to the same lethal dose of radiation. In the second experiments, again 540 mice in 3 groups were used. All experimental procedures above were duplicated, except that the magnesium pemoline dose employed this time was only 1% of the previous dose (0.7 mg/kg) for each animal. Daily mortality was observed and recorded up to 30 days after irradiation.

Results. Table I shows the post-irradiation survival percentage for 3 groups of the first experiment along with the standard deviations. In group I, 100% of the mice injected with magnesium pemoline were still alive on the 7th post-irradiation day while 15% of the tragacanth control animals had died. All the control mice were dead by the 14th day after exposure versus 60% of the magnesium pemoline mice. Sixteen days after being exposed to 750 R of X-irradiation 35% of the experimental mice survived, and no further mortality was observed up to 30 post-irradiation days. Almost the same pattern was observed in groups II and III, except that the magnesium pemoline animals started to die on the 6th and 5th post-irradiation days respectively for each group. None of the control mice in these groups were alive on the 13th day after exposure. On this same day, 30% of the magnesium pemoline mice in group II and 15% in group III still survived. By the 16th day, these percentages were reduced to 17% for group II and 5% for group III. No further mortality was observed up to 30 days.

Table II presents data obtained from the second experiment with the magnesium pemoline dose reduced to 1% of the dose administered in the previous experiment. On the 8th day after exposure, 74% of control mice in group I were still alive versus 100% of the experimental mice. All the tragacanth mice were dead 2 weeks after irradiation. At this time 40% of the magnesium pemoline mice still survived. This percentage was reduced to 30% on the 21st day, and after that no further mortality was observed. None of the control animals in group II lived after the 13th day while 25% of the experimental mice were still alive. On the 15th day, however, this score decreased to 8% and no more mortality was observed up to 30 days. There was no significant difference in mortality between magnesium pemoline and tragacanth mice in group III. All animals in this group died on the 14th post-irradiation day.

Discussion. As we see in both Table I and Table II, these 2 experiments confirm our earlier report regarding the long-term and short-term radioprotective effect of magnesium pemoline. The results consistently show that the drug was most effective when the time interval between drug administration and radiation exposure was the shortest. While there was no significant difference between the short-term protection in these 2 cases, there was no question about the effectiveness of the higher drug dose for the long-term protection.

Up to date, we have not been able to explain satisfactorily the mechanism of this protective effect of magnesium pemoline against ionizing radiations. The short-term protection was shown to be independent of the drug dose (group I, Tables I and II). For the long-term protection, however, the protective effect was more pronounced with higher drug dose (70 mg/kg). Even at this high dose, it is difficult to explain the effectiveness of magnesium pemoline 1 or 2 weeks after i.p. injection. The half-life of magnesium pemoline is about 14 h, and the total effect of the drug is almost negligible at drug dose as high as 20 mg/kg after 24–36 h. Comparing this to the dose employed in our second experimental part (0.7 mg/kg), one notes that magnesium pemoline still offered some degree of protection 1 week after its administration. There is no question that the animals were under certain physical strain during the

Table I. Post-irradiation % survival

Post-irradiation days	Group I		Group II		Group III	
	Magnesium pemoline	Tragacanth	Magnesium pemoline	Tragacanth	Magnesium pemoline	Tragacanth
1	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
2	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
3	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
4	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
5	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	98 ± 0.6
6	100 ± 0	94 ± 0.3	100 ± 0	96 ± 1.5	97 ± 1.0	96 ± 1.2
7	100 ± 0	85 ± 1.2	98 ± 0.4	85 ± 2.1	95 ± 2.0	78 ± 3.7
8	98 ± 2.0[a]	61 ± 2.7	93 ± 3.2	60 ± 3.4	85 ± 3.0	54 ± 3.9
9	94 ± 1.8	47 ± 3.5	85 ± 2.3	40 ± 2.6	76 ± 1.6	41 ± 2.3
10	82 ± 2.5	23 ± 2.0	65 ± 1.0	20 ± 3.0	54 ± 2.3	22 ± 3.1
11	66 ± 0.7	13 ± 1.3	44 ± 2.2	12 ± 1.7	33 ± 1.4	10 ± 0.2
12	57 ± 1.6	6 ± 0.7	38 ± 0.8	3 ± 1.3	19 ± 0	4 ± 0.7
13	46 ± 3.0	2 ± 0.4	30 ± 1.3	0	15 ± 2.5	0
14	40 ± 0.4	0	23 ± 0.5		9	
15	37 ± 1.6		20 ± 3.1		5	
16	34 ± 1.1		17 ± 0.7		.	
17	.		.		.	
.	.		.		.	
30	34		17		5	

Radiation dose: 750 R. Magnesium pemoline dose: 70 mg/kg. Tragacanth dose: 70 mg/kg. I.p. injection – irradiation interval: group I, 5 min, group II, 1 week, group III, 2 weeks. [a] Standard deviations.

Table II. Post-irradiation % survival

Post-irradiation days	Group I		Group II		Group III	
	Magnesium pemoline	Tragacanth	Magnesium pemoline	Tragacanth	Magnesium pemoline	Tragacanth
1	100	100	100	100	100	100
2	100	100	100	100	100	100
3	100	100	100	100	100	100
4	100	100	100	100	100	100
5	100	100	100	100	100	100
6	100	93 ± 0.6	100	97 ± 3.0	97 ± 2.3	97 ± 3.4
7	100	86 ± 1.9	94 ± 3.0	90 ± 1.6	94 ± 3.7	92 ± 0.2
8	100	74 ± 3.4	84 ± 0.6	77 ± 5.2	78 ± 1.7	70 ± 0.8
9	93 ± 2.3	57 ± 0.7	71 ± 4.5	57 ± 5.5	52 ± 3.5	52 ± 3.5
10	83 ± 1.6	40 ± 4.3	59 ± 1.2	39 ± 3.6	39 ± 4.6	40 ± 4.3
11	73 ± 3.4	23 ± 1.5	46 ± 5.0	25 ± 4.6	26 ± 0.3	23 ± 6.0
12	57 ± 4.5	10 ± 3.3	33 ± 2.7	12 ± 5.1	13 ± 1.2	8 ± 1.8
13	47 ± 0.2	5 ± 2.6	25 ± 3.3	0	6 ± 5.6	3 ± 0.6
14	40 ± 1.7	0	17 ± 3.5		0	0
15	40		8 ± 2.8			
16	40					
17	40					
18	37 ± 1.0					
19	37					
20	37					
21	30 ± 3.8					

No further mortality up to 30 days. Radiation dose: 750 R. Magnesium pemoline dose: 0.7 mg/kg. I.p. injection – irradiation interval: group I, 5 min, group II, 1 week, group III, 2 weeks.

irradiation. A number of investigators[2–4] have reported that pemoline, the parent compound of magnesium pemoline, improved the performance of fatigued human subjects. If this is the effect, it would contradict our recent consistent observations that magnesium pemoline, when injected immediately after irradiation, also exhibited significant protection[5]. It has been observed that animals pretreated with magnesium pemoline appeared to tolerate post-ictal depression better than control animals[6]; however the question of pretreatment or post-treatment of the animals with the drug is apparently not a critical one in our present experiments, since the post-treated mice too were protected from lethal doses of radiation.

We are in the process of determining the site of action of magnesium pemoline associated with this radioprotective effect by exposing various parts of the animal's body to radiation instead of giving them whole-body irradiation. When one can point out exactly this site of action of the drug, perhaps one may be able to relate the biochemical effect[7] of the drug to its protection against ionizing radiation. Magnesium pemoline, in addition to improving learning and memory, also enhances brain RNA polymerases[8]. If it is true that the increase in radiation resistance results from the action of the drug on the nervous system, it would be important to find out how much one can associate this protective effect with the mechanism by which the drug activates the nuclear aggregate enzymes responsible for RNA synthesis. It is quite interesting to note also that while other stimulants of the central nervous system such as methamphetamine and methylphenidate (Ritalin) did not produce the selective activation of the true RNA polymerase system, neither did they offer any degree of protection against ionizing radiation.

Since the potential application of magnesium pemoline in radiotherapy is quite possible, we are in the process of studying the effect of this drug (at low doses) on mice with Ehrlich ascites tumor treated with X-irradiation at various doses and dose rates[9,10].

Zusammenfassung. Magnesium-Pemoline, ein mildes Reizmittel für das Nervensystem, hat sich als gutes Strahlenschutzmittel für kurze oder lange Zeitdauer erwiesen. Für den Schutz über eine kurze Zeitdauer ist die Wirkung abhängig von der Arzneimitteldosis, für denjenigen über eine längere Zeitdauer (bis zu 2 Wochen) ist jedoch eine sehr hohe Dosis (75 mg/kg) notwendig.

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