Inhibition of RNA Synthesis in Mammalian Cells by Pentobarbital Sodium[1] (35088)

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In a previous paper we reported that the specific activity of total cellular RNA of Ehrlich ascites tumor cells exposed *in vivo* to or incubated *in vitro* with uridine-^{3}H remained constant between 10 min and 4 hr after exposure to the radioactive precursor (1). When Ehrlich ascites cells were prelabeled for 30 min with uridine-^{3}H and then incubated with actinomycin D, the specific activity of total cellular RNA decreased. However, no decrease in specific activity of total cellular RNA occurred when prelabeled cells were incubated under the same conditions with pentobarbital sodium. In addition, we reported that the concentration of pentobarbital used (1.5 mg/ml) produced a 97% inhibition of the incorporation of uridine-^{3}H into RNA of Ehrlich ascites cells. On the basis of these results we postulated that the RNA of Ehrlich ascites cells is pulse labeled by exposure to uridine-^{3}H and that the newly synthesized, rapidly labeled RNA is not appreciably broken down for at least 4 hr. On the contrary, actinomycin D causes a breakdown of rapidly labeled RNA, a finding also supported by other investigators (2, 3).

Our interpretation rested on the assumption that pentobarbital is an inhibitor of RNA synthesis, an assumption only supported by our finding that the incorporation of uridine-^{3}H into RNA of Ehrlich ascites cells was in fact largely inhibited by the doses of pentobarbital used in our experiments. However, Nakata and Bader (4) have shown that a reputed inhibitor of RNA synthesis, 2-mercapto-1-(β-4-pyridylethyl) benzimidazole (MPB) does not actually inhibit RNA synthesis but simply inhibits the uptake of nucleosides into cells. When RNA synthesis was measured by the incorporation of ^{32}P into RNA, MPB was not inhibitory (4). This possibility has substantial implications, because, if pentobarbital does not inhibit RNA synthesis, then the constant specific activity of total cellular RNA between 10 min and 4 hr after exposure to uridine-^{3}H could also be explained by the breakdown of newly synthesized RNA and the prompt reutilization of its breakdown products. On the other hand, if pentobarbital is an inhibitor of RNA synthesis then one must seriously consider the possibility that treatment with actinomycin D causes breakdown of RNA thus introducing a disturbing artifact in a number of experiments reported in the literature.

The purpose of this note is to report that pentobarbital sodium inhibits the incorporation of ^{32}P and sodium formate-^{14}C into nucleic acids of Ehrlich ascites cells and to confirm our previous conclusion that actinomycin D very likely causes a breakdown of RNA rapidly labeled with uridine-^{3}H.

Methods. Fels A female mice, bred in this laboratory, were used throughout these experiments. Ehrlich ascites tumor cells were maintained by weekly serial transfer. The tumor, a hypotetraploid subline, has been previously described in detail (5). For *in vitro* experiments 2.5×10^7 Ehrlich ascites tumor cells pooled from mice bearing 5-day-old tumors were suspended in a 7:2 mixture of Earle's balanced salt solution and newborn

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calf serum to which the desired radioactive precursor was added. The cells were incubated at 37° in an Erlenmeyer flask in an atmosphere of 95% air and 5% CO_2 in a humidified incubator. The incubation was terminated by pipetting an aliquot of the incubation mixture directly into 1 vol of cold 1 *N* percholoric acid. For *in vivo* experiments Fels A male mice were inoculated with approximately 12×10^6 tumor cells intraperitoneally. On the 5th day after inoculation the mice were injected intraperitoneally with ^{32}P as described in detail in the tables. The specific activities of RNA and DNA were determined by the method of Scott *et al.* (6) as modified by Hinrichs *et al.* (7). Phosphoric acid, $^{32}PO_4$ carrier-free, in water was purchased from New England Nuclear Corp. Uridine-^{3}H generally labeled (4.5 Ci/mmole) and sodium formate-^{14}C (41.7 mCi/mmole) were purchased from Amersham Searle, Arlington Heights, Illinois. Pentobarbital sodium was a gift of Dr. James Price, Abbott Laboratories, North Chicago, Illinois.

Results. Table I shows that pentobarbital inhibits the incorporation of sodium formate-^{14}C into RNA of Ehrlich ascites cells incubated *in vitro*. At a concentration of 1 mg/ml of pentobarbital the incorporation of sodium formate-^{14}C into RNA is inhibited 85%. Table II shows that pentobarbital inhibits the incorporation of ^{32}P into nucleic acids of Ehrlich ascites cells growing in the peritoneal cavity of mice, and that, at the dose of pentobarbital used, incorporation of ^{32}P into both RNA and DNA is inhibited more than 50%. Table III shows two different experiments. In Expt. 1, the cells were preincubated with pentobarbital and then incubated with ^{32}P. At the maximum concentration of 1.5 mg/ml of pentobarbital the incorporation of ^{32}P into both RNA and DNA was inhibited approximately 85%. In Expt. 2 the cells were preincubated with ^{32}P for 30 min and then for another additional 30 min with pentobarbital. This experiment shows that the addition of pentobarbital inhibits the further incorporation of ^{32}P into nucleic acids.

TABLE I. Effect of Pentobarbital on the Incorporation of Sodium Formate-^{14}C into RNA of Ehrlich Ascites Cells.[a]

Pentobarbital conc (mg/ml)	dpm in RNA/mg RNA $\times 10^{-3}$ (mean in parentheses)	Inhibition (%)
0	45-51-44-51-48-41 (47)	0
0.10	22-25-21 (23)	51
0.15	20-19-22 (20)	56
0.25	18-21-17 (19)	60
0.50	17-13-11 (13)	71
1.00	3-10-8 (7)	85

[a] Freshly harvested cells, preincubated for 15 min in pentobarbital or solvent, were incubated for 30 min with 10 μCi/ml of sodium formate-^{14}C. Incubation conditions and RNA determinations are described in Methods and Materials.

TABLE II. Effect of Pentobarbital on the *in Vivo* Incorporation of ^{32}P into Nucleic Acids of Ehrlich Ascites Cells.[a]

Treatment	cpm/mg RNA (mean) $\times 10^{-3}$	cpm/mg DNA (mean) $\times 10^{-3}$
Solvent only	84-67-76-56 (70.8)	15-12-11-12 (12.5)
Pentobarbital	32-39-26 (32.3)	5-7-6 (6.0)

[a] Mice, 5 days after intraperitoneal inoculation of Ehrlich tumor were injected intraperitoneally with 2 mg of pentobarbital, dissolved in 0.9% NaCl. (Controls received solvent only.) Ten min later, all mice were injected with 60 μCi/mouse of ^{32}P, and they were killed after another 30 min. Nucleic acids were determined as described in Methods and Materials.

These results then seem to indicate that pentobarbital, under the conditions used, inhibits the synthesis of RNA in Ehrlich ascites cells. Even if pentobarbital is a *bona fide* inhibitor of RNA synthesis, the finding that the specific activity of total cellular RNA after prelabeling with uridine-^{3}H remains constant in the presence of pentobarbital (1) could also be explained as due to the fact that the natural breakdown of rapidly labeled RNA, as well as its synthesis, is inhibited by the drug. This alternative is also suggested by recent findings that barbiturates prolong the half-life of ribosomal RNA (8) and inhibit RNase activity in rat liver (9). To investigate this possibility we have prela-

TABLE III. Effect of Pentobarbital on the Incorporation of ^{32}P into Nucleic Acids of Ehrlich Ascites Cells.[a]

Expt.	Treatment	cpm/mg RNA (mean) $\times 10^{-3}$	cpm/mg DNA (mean) $\times 10^{-3}$
1[b]	Solvent only	92–95 (93.5)	16–21 (18.6)
	Pentob., 0.5	33–49 (40.7)	3–7 (5.0)
	1.0	18–21 (19.5)	1.5–2.4 (2.0)
	1.5	14–15 (14.5)	3.2–1.6 (2.4)
2[b]	Solvent only	156–175 (166)	53–70 (62)
	Pentob., 0.5	95–89 (92)	39–29 (34)
	1.0	77–80 (78.5)	24–25 (24.5)
	1.5	69–56 (62.5)	20–20 (20)

[a] Ehrlich ascites cells harvested on the 5th day of growth. Incubation conditions and determination of nucleic acids are described in Methods and Materials. Pentobarbital concentration is expressed in mg/ml. ^{32}P concentration was 20 μCi/ml.

[b] In Expt. 1, the cells were preincubated in pentobarbital (or solvent) for 15 min and then incubated for 30 min with ^{32}P; in Expt. 2, the cells were preincubated with ^{32}P for 30 min, then, after addition of pentobarbital (or solvent) the incubation in the presence of ^{32}P was continued for another 30 min.

beled Ehrlich ascites cells with uridine-^3H for 30 min and then divided them into four groups, namely: (i) control; (ii) cells incubated with actinomycin D, 10 μg/ml; (iii) cells incubated with pentobarbital, 1.5 mg/ml; and (iv) cells incubated with both pentobarbital and actinomycin D. The results shown in Fig. 1 indicate that while the specific activity of total cellular RNA remains constant in the presence of pentobarbital, it decreases when cells are incubated with either actinomycin D only, or with actinomycin D plus pentobarbital.

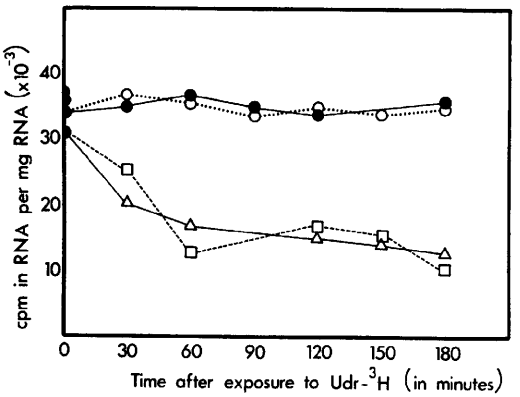

FIG. 1. Effect of pentobarbital and actinomycin D on the specific activity of RNA of Ehrlich ascites cells prelabeled with uridine-^3H. All cells were incubated for 30 min with uridine-^3H (1.0 μCi/ml) then divided into 4 groups to which inhibitors were added as follows: (●—), controls with solvent only; (○ . .), pentobarbital, 1.0 mg/ml; (△—), actinomycin D, 10 μg/ml; (□ - -), pentobarbital, 1.0 mg/ml plus actinomycin D, 10 μg/ml. The cells were then incubated for the intervals indicated on the abscissa and the specific activity of RNA determined as described in Methods and Materials.

Discussion. The present results indicate that pentobarbital inhibits both *in vivo* and *in vitro* the incorporation of ^{32}P and sodium formate-^{14}C into nucleic acids of Ehrlich ascites tumor cells. The results with DNA confirm previous results by Baserga and Weiss (10). It seems, therefore, that pentobarbital sodium may be considered under certain conditions as an inhibitor of both RNA and DNA synthesis, at least in Ehrlich ascites tumor cells. This does not exclude that pentobarbital may also inhibit the uptake of nucleosides into cells. In fact, preliminary experiments (not shown here) do indicate that in the presence of pentobarbital the uptake of uridine-^3H into Ehrlich ascites cells is also decreased. However, the effect of pentobarbital sodium on the incorporation of ^{32}P and sodium formate-^{14}C into RNA seems to indicate that pentobarbital also has a direct action on the synthesis of RNA. If pentobarbital is then considered a *bona fide* inhibitor of RNA synthesis then the interpre-

tation of the results presented in our previous paper (1) is confirmed, namely: (i) that the RNA of Ehrlich ascites cells is pulse labeled by exposure to uridine- ^{3}H; (ii) that no appreciable breakdown and subsequent re-utilization of breakdown products occurs in the rapidly labeled RNA of these Ehrlich ascites cells; and (iii) that actinomycin D causes an artificial breakdown of rapidly labeled RNA.

Summary. Pentobarbital inhibits the incorporation of ^{32}P and sodium formate-^{14}C into RNA of Ehrlich ascites tumor cells *in vivo* and *in vitro*. In addition, the RNA specific activity of Ehrlich ascites cells prelabeled with uridine-^{3}H, remains constant in untreated cells and in cells treated with pentobarbital, but decreases in cells treated with actinomycin D or with actinomycin D plus pentobarbital.

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