MOLECULAR PHARMACOLOGY, 7, 484–489

SHORT COMMUNICATION

Stability of Polyribosomes Isolated from Rat Liver after Phenobarbital Administration

A. M. COHEN[1] AND R. W. RUDDON[2]

Departments of Pharmacology and Oral Biology, The University of Michigan Medical and Dental Schools, Ann Arbor, Michigan 48104

(Received March 27, 1971)

SUMMARY

The effect of daily injections of phenobarbital (75 mg/kg) on the degradation of rat liver polyribosome suspensions during storage *in vitro* at 0–4° was examined. Sucrose gradient analysis of polysomes isolated from animals treated for 3–5 days with 0.9% NaCl revealed that after 24–48 hr of storage at 0–4° a disaggregation of large polysome species occurred, together with an increase of 50–85% in the number of monomer and dimer species. In contrast, polyribosomes derived from rats treated with phenobarbital for 3–5 days were shown to be almost completely resistant to degradation under the same conditions.

In a previous report from this laboratory (1), evidence was presented which indicated that an enhanced incorporation of orotic acid-^{3}H into cytoplasmic ribosomal RNA was one of the earliest detectable effects produced in rat liver after phenobarbital administration. This increased labeling of rRNA did not appear to be due to an elevated synthesis of rRNA, but to an alteration of rRNA processing or stability. Since a drug-induced decrease in the degradation of cytoplasmic ribosomal RNA could be one of the explanations for these observations, a study of the effects of phenobarbital on the stability of hepatic polyribosomes was undertaken. In this paper evidence is presented which shows that (a) when hepatic polysomal preparations are stored in sucrose-free buffer at 0–4° breakdown to monomer and dimer species occurs, and (b) polysomes isolated from rats treated with phenobarbital are resistant to the degradation *in vitro* that is observed with polysomes derived from animals treated with 0.9% NaCl.

Male Sprague-Dawley rats (Spartan Farms, Lansing, Mich.) weighing 60–70 g were given single daily intraperitoneal injections of either phenobarbital (75 mg/kg) dissolved in 0.9% NaCl or 0.9% NaCl alone. The period of treatment ranged from 3 to 5 days, depending on the experiment. Some of the rats were fasted for 24–48 hr after the last injection before being killed by decapitation. In one experiment a group of animals treated with 0.9% NaCl was allowed continuous access to food in order to determine the effects of fasting alone on subsequent polysome degradation *in vitro*. In all experiments the animals were killed between 8 and 9 a. m. to avoid any diurnal variation. For each experiment the livers from two or three animals in

[1] Predoctoral Fellow, supported by the National Institute of Mental Health (5 F01 MH 37949). Present address, Division of Pharmacology, Sloan-Kettering Institute for Cancer Research, New York, New York 10021.

[2] Research supported by Grant NIH-DE-02731 from the National Institutes of Health. Address requests for reprints to R. W. Ruddon, Department of Oral Biology, Pharmacology Section, The University of Michigan, School of Dentistry, Ann Arbor, Michigan 48104.

each treatment group were pooled after death.

Total hepatic polyribosomes (i.e., free and membrane-bound polyribosomes) were isolated according to the procedure of Wettstein, Staehelin, and Noll (2). Livers were quickly removed, weighed, and pooled in 2 volumes of ice-cold 0.25 M sucrose in 0.05 M Tris-HCl (pH 7.5)–0.025 M KCl–0.005 M $MgCl_2$ (TKM). All subsequent operations were performed at 0–4°. Livers were homogenized in a glass homogenization tube fitted with a motor-driven Teflon pestle at 1400 rpm, using 20 strokes. The resulting homogenate was centrifuged at 12,500 × *g* for 10 min in a Sorvall refrigerated centrifuge to yield a postmitochondrial supernatant fraction, which was subsequently made 1.2% (w/v) with respect to sodium deoxycholate. An aliquot (2.5 ml) of this suspension was then layered over a discontinuous sucrose density gradient composed of 5 ml of 0.5 M sucrose in TKM on top of 4 ml of 2.0 M sucrose in TKM. The gradients were centrifuged at 40,000 rpm in a Spinco 50 titanium rotor on a model L2-65B Spinco ultracentrifuge for at least 16 hr to ensure complete sedimentation of all polyribosome species (3). After centrifugation, the sucrose layers were removed by vacuum suction, leaving an amber pellet at the bottom of the tube. The surface of this pellet and the walls of the centrifuge tube were gently rinsed with several volumes of 0.25 M sucrose in TKM. The rinsed pellet was scraped from the bottom of the tube and placed in a small glass homogenizing tube containing ice-cold TKM buffer, and then resuspended by means of a

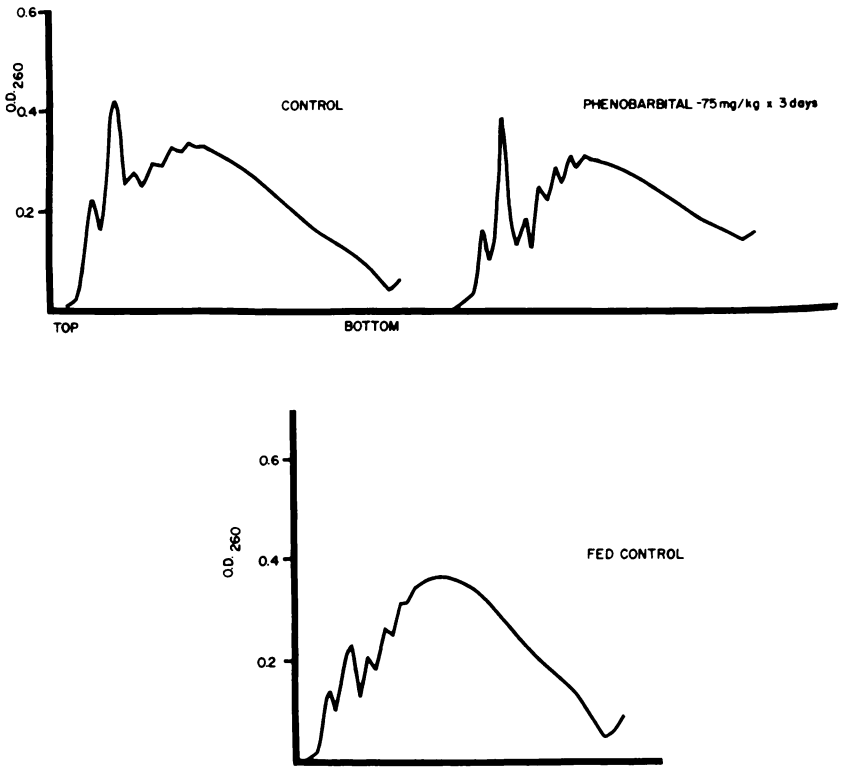

FIG. 1. *Sucrose density gradient analysis of freshly prepared hepatic polyribosomes*

Samples of hepatic polyribosomal protein (1.5 mg) isolated from postmitochondrial supernatant fractions from fasted control, fasted phenobarbital-treated, and fed control rats were layered over linear 10–35% (w/v) sucrose gradients in TKM (0.025 M KCl, 0.005 M $MgCl_2$, and 0.05 M Tris-HCl, pH 7.5). Gradients were centrifuged at 22,000 rpm in the Spinco SW 27 rotor for 2.5 hr at 0°. Tubes were then punctured, and the absorbance of the effluent at 260 nm was recorded.

loosely fitting glass pestle. The suspension of polysomes was analyzed for protein content by the method of Lowry *et al.* (4).

The polysomal preparation (1.5 mg of protein) was layered on top of a 10–35% (w/v) linear sucrose gradient made up in TKM. The gradients were then centrifuged at 22,000 rpm for 2.5 hr in a Spinco SW 27 rotor and allowed to come to a stop without braking. After centrifugation, the tubes containing the gradients were punctured, and the resulting effluent was allowed to pass through a flow cell adapter in a Beckman DB recording spectrophotometer set to record at 260 nm. The total polysome area as well as the area of the monomer plus dimer peaks of the resulting polysome profile were quantitated by means of a compensating polar planimeter. Determinations were made on the freshly suspended polysomes as well as on the same preparation allowed to remain in TKM buffer at 0–4° for periods up to 48 hr. The percentage change in the area of the monomer plus dimer peaks as compared to the total polysome area was used as an indication of polysome disaggregation during the storage period.

The stability of isolated rat liver polyribosomes is affected by a variety of environmental influences. Many studies have indicated that they are extremely labile to ribonuclease activity, which rapidly degrades them to species containing no more than one or two ribosomes per cluster (5–7). Wettstein *et al.* (2) have reported that incubation of polysomes at 37° in buffer alone results in disaggregation of larger polysome species, with the accumulation of oligosomes. Pron-

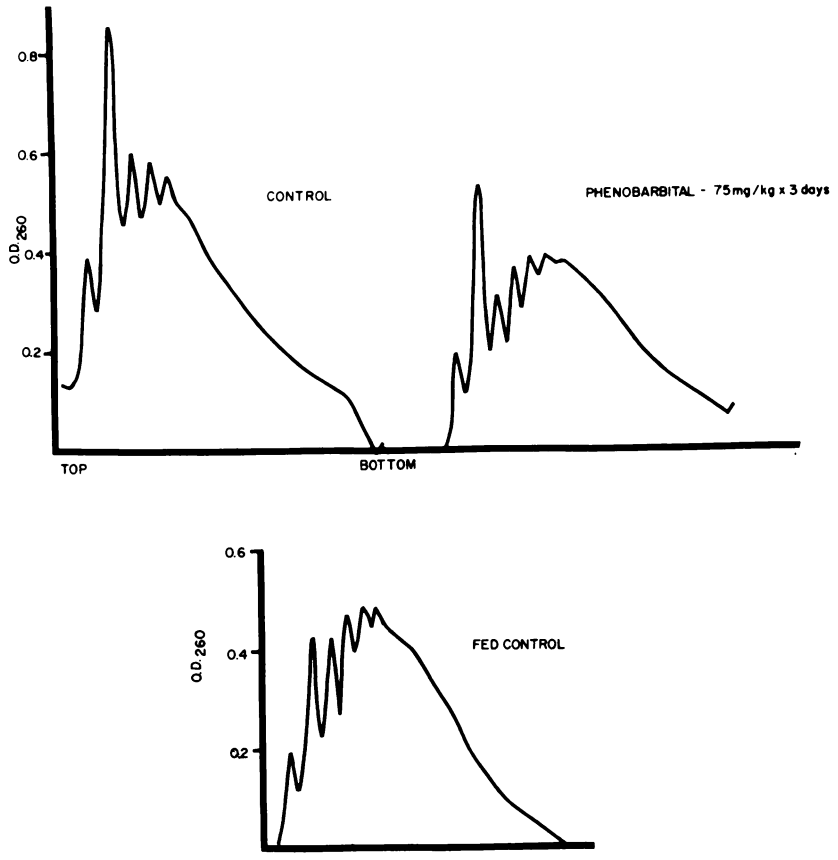

FIG. 2. *Sucrose density gradient analysis of hepatic polyribosomes after storage for 24 hr*
Conditions were the same as in Fig. 1, except that polyribosomes were allowed to remain suspended in TKM buffer at 0–4° for 24 hr prior to analysis on sucrose gradients.

czuk *et al.* (8) have shown that polysomes suspended in buffer will disaggregate when incubated with ATP or other nucleoside triphosphates even in the absence of the factors necessary for protein synthesis. This effect of the nucleoside triphosphates is antagonized by Mg^{2+}. Therefore, polysomal breakdown *in vitro* does not appear to be necessarily linked to the process of protein synthesis. In our laboratory it has been observed that polysomes derived from the livers of untreated rats or rats treated with 0.9% NaCl will disaggregate to oligosomes when allowed to remain suspended for prolonged periods in sucrose-free buffer at 0–4°. Accordingly, we have used this observation to examine the effects of prior treatment of rats with phenobarbital on the subsequent stability of the isolated hepatic polyribosomes.

In the first series of experiments, rats received three consecutive daily injections of either phenobarbital, 75 mg/kg dissolved in 0.9% NaCl, or 0.9% NaCl alone. After receiving the last injection, the rats were fasted for 24 hr, and a total hepatic polysome fraction was prepared by the deoxycholate method. As a control for the effects of fasting on polysome stability, one group of rats treated with 0.9% NaCl was allowed access to food throughout the entire study. Figure 1 shows the hepatic polysome profiles from the control, phenobarbital-treated, and fed control groups when analyzed on linear sucrose gradients as soon as possible (3 hr) after their resuspension in pH 7.5 buffer. Fasting alone resulted in a degree of polysome breakdown, as evidenced by the increased area of the monomer and dimer peaks in the control and phenobarbital groups as compared to the fed control group. Figure 2 shows the distribution pattern of these same polysome preparations after they had remained suspended in buffer at 0–4° for 24 hr. It is apparent from these profiles that a loss of heavier polysomal aggregates with a concomitant accumulation of oligosomes occurred during the prolonged storage of polyribosomes isolated from the livers of both the fasted and fed control rats. In contrast, the polysomes derived from rats treated with phenobarbital remained intact under the same conditions. These results were confirmed in a second experiment, in which rats received five daily injections of either phenobarbital or 0.9% NaCl and were fasted for the 48 hr between the fourth injection and the time of death (Figs. 3 and 4). Figure 3 shows that the distribution patterns of the polyribosomes from both the phenobarbital- and 0.9% NaCl-treated animals were similar when analyzed shortly after they were resuspended in buffer. However, when these same polysomes were stored for 24 hr at 0–4° and then analyzed on linear sucrose gradients, a marked difference was observed. As can be seen in Fig. 4, storage of the polyribosomes isolated from animals given 0.9% NaCl alone resulted in an almost

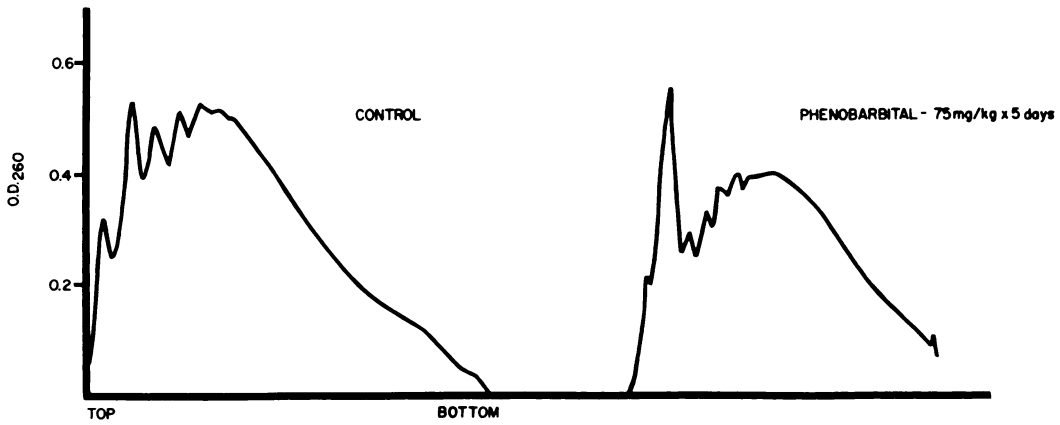

FIG. 3. *Sucrose density gradient analysis of freshly prepared hepatic polyribosomes from control and phenobarbital-treated rats fasted for 48 hr prior to death*

Conditions were the same as in Fig. 1.

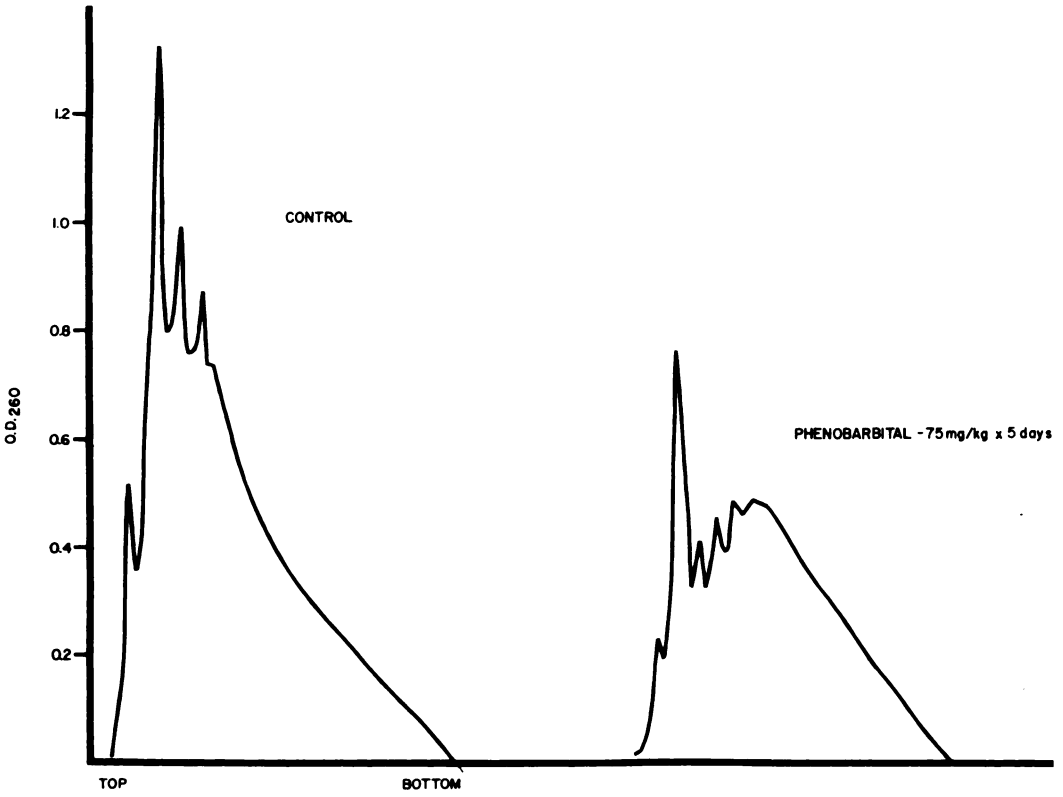

FIG. 4. *Sucrose density gradient analysis of stored polyribosomes from control and phenobarbital-treated rats fasted for 48 hr prior to death*

Conditions were the same as in Fig. 1, except that polyribosomes were allowed to remain suspended in TKM buffer at 0–4° for 24 hr prior to analysis on sucrose gradients.

complete loss of heavy polysomal material and the accumulation of species consisting of four ribosomes per cluster or less. Analysis of the areas under these curves revealed a 51% increase in the monomer plus dimer area during this period. However, the preparation of polyribosomes derived from animals treated with phenobarbital remained essentially intact. This was evidenced by the presence of a substantial amount of heavier polysome species and an increase of only 12% in the monomer plus dimer area during the 24 hr of storage. The addition of 0.5 mM phenobarbital to polyribosomes prepared from 0.9% NaCl-treated rats did not prevent their breakdown. It should be noted that the enhanced stabilization of polysomes from animals treated for 5 days with phenobarbital was also present, but less evident, when the polysomal preparations were incubated at 37° for 20 min.

These results suggest that a portion of the enhanced stability of polyribosomes derived from phenobarbital-treated rats may result from a decreased content of ribonuclease activity in the polysome suspensions from the barbiturate-treated animals. However, attempts to demonstrate the presence of ribonuclease activity in polysomal preparations by means of adding exogenous ^{3}H-labeled rat liver RNA were unsuccessful. It is likely that polysome-associated RNase is not readily accessible to exogenously added substrate. A similar observation was made by Eker *et al.* (9), who demonstrated that polysomes were able to degrade endogenous, radioactively labeled RNA but not exogenously added material.

Although ribonuclease activity may be associated with isolated rat liver polysomes, the origin of such nuclease activity is unknown. During the isolation of polyribo-

somes with sodium deoxycholate, both lysosomal and microsomal membranes are destroyed. It is conceivable, therefore, that ribonuclease activity formerly present in these organelles is released and subsequently becomes associated with the polyribosomes. The recent report by Louis-Ferdinand and Fuller (10) of a marked reduction of microsomal ribonuclease activity in the livers of rats treated with phenobarbital suggested to us that it might be possible to isolate intact polyribosomes directly from the microsomal fraction of drug-treated animals. Accordingly, an attempt was made to isolate intact polyribosomes from relatively lysosome-free microsomes (11) rather than from the postmitochondrial supernatant fraction. This procedure was followed in order to eliminate the ribonuclease inhibitor of rat liver cell sap as a factor controlling polysome stability. However, it was not possible to recover intact polysomes from the microsomal fraction in the absence of cell sap, even after five consecutive daily injections of phenobarbital. Evidently, sufficient ribonuclease activity remained in the microsomes even after prolonged treatment with phenobarbital to cause complete disruption of polysome integrity. The control of polysomal aggregation and disaggregation is a complicated and poorly understood process, however, and involves several factors in addition to ribonuclease activity (8, 12, 13). Thus, it is unclear at the present time by what mechanism phenobarbital enhances the stability of hepatic polyribosomes stored *in vitro* and whether this is indicative of a change *in vivo* in the functional capacity of the protein-synthesizing machinery.

ACKNOWLEDGMENT

The authors wish to thank Mrs. Cynthia Rainey for help in the preparation of the manuscript.

REFERENCES

1. A. M. Cohen and R. W. Ruddon, *Mol. Pharmacol.* **6**, 540 (1970).
2. F. O. Wettstein, T. Staehelin and H. Noll, *Nature* **197**, 430 (1963).
3. G. Blobel and V. R. Potter, *J. Mol. Biol.* **26**, 279 (1967).
4. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
5. G. Blobel and V. R. Potter, *Proc. Nat. Acad. Sci. U. S. A.* **55**, 1283 (1966).
6. H. R. Mahler and B. J. Brown, *Arch. Biochem. Biophys.* **125**, 387 (1968).
7. P. R. Adiga, R. V. Hussa, M. C. Robertson, H. R. Hohl and T. Winnick, *Biochemistry* **60**, 606 (1968).
8. A. W. Pronczuk, B. S. Baliga and H. N. Munro, *Biochem. J.* **110**, 783 (1968).
9. P. Eker, P. Weidenmuller and A. Pihl, *FEBS Lett.* **9**, 17 (1970).
10. R. T. Louis-Ferdinand and G. C. Fuller, *Biochem. Biophys. Res. Commun.* **38**, 811 (1970).
11. O. A. Scornik, M. B. Hoagland, L. C. Pfefferkorn and E. A. Bishop, *J. Biol. Chem.* **242**, 131 (1967).
12. A. Fleck, J. Shepherd and H. N. Munro, *Science* **150**, 628 (1965).
13. W. H. Wunner, J. Bell and H. N. Munro, *Biochem. J.* **101**, 417 (1966).