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MICROSOMAL NUCLEIC ACID BREAKDOWN IN LIVERS OF PHENOBARBITAL-TREATED RATS*

MARY J. MYCEK

Department of Pharmacology, New Jersey College of Medicine and Dentistry, Newark, N.J., U.S.A.

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Abstract—Endogenous ribonuclease activity has been determined in the microsomal fractions isolated from phenobarbital-treated and saline-treated rat livers. Drug administration (10 mg/100 g) daily for 1, 2 and 4 days results in a progressive inhibition of ribonuclease activity as compared to control rats receiving physiological saline intraperitoneally. The decrease in ribonuclease activity parallels both the induction of *O*-demethylase and development of tolerance to the hypnotic effects of barbiturates. Ribonuclease activity was established by comparing spectrophotometric data with radioactivity results from microsomal fractions obtained from animals receiving a pulse label of 5 μc/100 g of orotic acid-6-^{14}C prior to subsequent treatment with drug or saline. The polycyclic hydrocarbon, 3-methylcholanthrene (2·5 mg/100 g), had no effect on ribonuclease activity when administered daily for 4 days. Several explanations for the lowered enzymatic activity are discussed.

CHRONIC treatment of rats with a variety of agents, including phenobarbital, results in the enhancement of the activity of an NADPH-requiring oxidative enzyme system localized in the liver microsomes.[1] This increase in enzymatic activity is dependent on protein synthesis as revealed by experiments *in vivo*[2] and *in vitro*,[3] and by experiments with inhibitors of protein synthesis.[4,5]

It has been suggested[6] that the increased protein synthesis is effected through a stimulation of messenger RNA formation. This hypothesis is supported by findings that RNA levels in the liver microsomes rise in phenobarbital-treated animals[7] and that increased levels of RNA polymerase activity have been observed in rat liver nuclei after 3-methylcholanthrene treatment,[6] which involved a related oxidative enzyme induction system.

At the present time, however, the possibility cannot be excluded that the oxidative drug-metabolizing activity may be altered, not only through an augmentation of messenger RNA levels by increased synthesis, but also through a decrease in the breakdown of an RNA species, either by reduced levels of intracellular ribonuclease activity or by inhibition of the catabolism. Indeed, treatment of adrenalectomized rats with cortisone produces lowered hepatic nucleic acid catabolism.[8] A similar result has been reported for regenerating rat liver.[9]

The studies in this communication deal with the influence of chronic phenobarbital administration on endogenous RNA breakdown in rat liver. They provide evidence that, as the level of the mixed function oxidase activity rises in the liver, producing tolerance of the animal toward the hypnotic effects of barbiturates, the breakdown of

* A preliminary report of this work was presented at the meetings of the Federation of American Societies for Experimental Biology, in Chicago, April 1967.

RNA in the microsomal fraction is depressed as compared to that observed in the same tissue fraction from nontolerant controls.

MATERIALS AND METHODS

Young adult male albino rats (90–150 g) of the Wistar strain obtained from the Marland Animal Farms, Wayne, N.J., were used. Routinely, animals were fasted for 18 hr before sacrifice.

Hemoglobin substrate was purchased from Worthington Biochemical Corp. Yeast RNA was a commercial grade product of Sigma Chemical Company, phenobarbital sodium was obtained from Merck and Company, and hexobarbital sodium was a product of Sterling-Winthrop. Orotic acid-6-^{14}C (22·6 mc/m-mole) was purchased from Calbiochem.

Isolation of subcellular components. Animals were decapitated, the livers were removed, rinsed in 0·25 M sucrose, blotted and homogenized in 0·25 M sucrose (1 : 10). All operations were carried out in the cold room at 5°. Microsomes and ribosomes were isolated from the homogenates by the differential centrifugation procedure.[10] Each liver was handled separately, except when *O*-demethylase activity was to be measured in parallel. In those experiments, two livers were combined and one-half of each was homogenized, as cited above, to obtain the microsomal fraction. The other half portion was homogenized according to Netter and Seidel,[11] and the supernatant fluid, after the separation of the mitochondrial fraction, was used for determination of *O*-demethylase. All fractions were frozen until assayed; they were thawed only once.

Incorporation of orotic acid-6-^{14}C. The labeled pyrimidine precursor with a specific activity of 22·6 mc/m-mole was diluted to contain 50 μc/ml. Each rat received one injection (5 μc/100 g) intraperitoneally 2 hr prior to subsequent administration of saline or phenobarbital. The liver components were isolated from the 0·25 M sucrose homogenate as indicated above. Radioactive measurements were carried out on samples containing 1 mg tissue protein dissolved in 1 ml dimethylsulfoxide, to which 7 ml ethanol and 10 ml toluene scintillator mixture were added.[12] Counting was performed by a Packard model 574 scintillation counter at 35 per cent efficiency.

Isolation of RNA. RNA was isolated from the microsomal fraction by the phenol extraction procedure described by Barlow and Mathias.[13]

Sleeping time. The hypnotic response to barbiturates was estimated by administration of hexobarbital (10 mg/100 g) intraperitoneally. The time elapsed from loss of the righting reflex to its recovery was considered as the duration of the response.

Ribonuclease assay. To estimate endogenous RNA breakdown, the following components were incubated at 38° for 30 min in a final volume of 2·5 ml: 2 ml of 1·5% denatured hemoglobin dissolved in 0·025 M tris buffer (pH 8) and 0·18 M NaCl, 0·5 ml of 0·2 M tris buffer (pH 8), and tissue fraction corresponding to 1 mg protein. The reaction was stopped by the addition of 4 ml of 10% trichloroacetic acid. Optical density of the filtrate was measured at 260 mμ against a suitable blank. After subtraction of the zero time values, the results were expressed as optical density per milligram of protein per 30 min. Protein was determined by the method of Lowry *et al.*[14] with bovine serum albumin as standard.

For the estimation of exogenous RNA catabolism, 0·02 g of yeast RNA was dissolved in 20 ml of 0·01 M acetate buffer (pH 5·2) and dialyzed vs. the same solvent

overnight at 0°. This served as a stock solution, aliquots of which were added to the above assay system.

O-demethylase assay. The *O*-demethylation of *p*-nitroanisole was followed by the formation of the chromogenic product, *p*-nitrophenol, according to the method of Netter and Seidel.[11]

RESULTS

Presence of ribonuclease activity in liver microsomes and nature of the product

Roth[15] has reported the presence of an RNase activity in mammalian microsomes and characterized it as fairly heat stable, with a pH optimum of 7·5–8·0, stimulated by high ionic strength, and inhibited by a cell sap component and by Mg^{2+}. The presence of such an activity has been confirmed in the present investigation, using the appearance of material absorbing at 260 mμ in the acid-soluble filtrate as an assay. A representative experiment illustrating the stimulatory activity of high ionic strength (i.e. 0·18 M NaCl) is shown in Fig. 1. The presence of exogenous protein, either serum albumin or

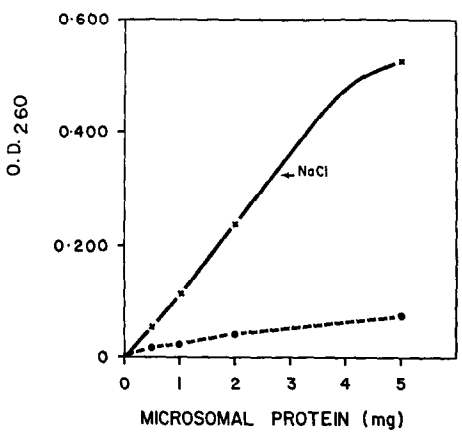

FIG. 1. Release of 260 mμ absorbing material from hepatic microsomal fraction as a function of microsomal protein and the presence of NaCl. Conditions of incubation are described under Materials and Methods. ×—×, 0·18 M NaCl present; ●- - -●, NaCl absent.

denatured hemoglobin, in the incubation medium promotes the release of the acid-soluble 260 mμ absorbing material, but the nature of this stimulation is undetermined at present. It is unlikely that the protein undergoes any significant degradation under the incubation conditions because the 260:280 ratio is not affected by the presence of protein (or its absence). With or without added protein, the optimum absorption of the acid-soluble product is at 260 mμ. The product (or products) of this reaction remains to be identified.

The reaction is dependent on the amount of microsomal protein (Fig. 1), on the duration of incubation, and on the temperature. Exposure of the tissue fraction to 100° for 10 min results in a loss of activity.

In agreement with the work of Roth,[16] we have also observed an inhibition of ribonuclease activity upon addition of the soluble fraction of liver homogenate to the

incubation. Dialysis of the cell sap does not alter its capacity to inhibit; however, boiling of this fraction does destroy its inhibitory activity.

Addition of Mg^{2+} (1×10^{-3} M) to the incubation system also inhibits the release of the acid-soluble 260 mμ absorbing material.

Effect of chronic phenobarbital administration on ribonuclease activity

Table 1 represents the RNase activity in the hepatic microsomes obtained from animals that had received phenobarbital intraperitoneally for 4 days compared to the activity found in the same tissue fractions from animals that had been injected with

TABLE 1. EFFECT OF PHENOBARBITAL TREATMENT ON RIBONUCLEASE ACTIVITY IN HEPATIC MICROSOMAL FRACTION

Treatment*	No. of animals	Body wt. (g)	Liver wt. (g)	Microsomal protein (mg/g liver)	O.D.$_{260}$/mg protein/30 min
Control (saline-injected)	10	144 ± 12·8	4·98 ± 0·38	15·7 ± 0·9	0·180 ± 0·008
Phenobarbital-treated	10	141 ± 9·1†	5·83 ± 0·37†	18·0 ± 0·5†	0·084 ± 0·008‡

* Rats were injected with either 0·9% NaCl or 10 mg/100 g of phenobarbital Na (i.p.) for 4 consecutive days. After having been starved overnight, they were sacrificed on the fifth day. Assay conditions are those described under Materials and Methods. The values represent the means and standard errors.

† Not significantly different from controls.

‡ P = 0·001 difference from controls.

physiological saline for the same period of time. Treatment with the drug produces a significant (53·3 per cent) inhibition in the release of the 260 mμ absorbing acid-soluble product from the liver microsomes. The lowering of enzymatic activity is also demonstrable when these data are expressed on a per gram of liver basis or as a function of the ratio of liver weight to body weight. In this latter instance, calculated correlation coefficients were not significant. No effect on the RNase activity associated with the ribosomes obtained from similarly treated animals was observed (Table 2).

TABLE 2. EFFECT OF PHENOBARBITAL TREATMENT ON RIBONUCLEASE ACTIVITY IN HEPATIC RIBOSOMES

Treatment*	No. of animals	O.D.$_{260}$/mg protein/30 min (mean + S.E.)	
Control (saline-injected)	6	0·394 ± 0·069	
Phenobarbital-treated	6	0·435 ± 0·055	N.S.

* Rats were injected with either 10 mg/100 g of phenobarbital Na or 0·9% NaCl (i.p.) for 4 consecutive days. After having been starved overnight, they were sacrificed on the fifth day. Assay conditions are those described under Materials and Methods. N.S. = not significant.

The possibility that phenobarbital itself might be the inhibitor of the breakdown reaction was eliminated when the drug produced no inhibition upon addition at several concentrations (i.e. 0·01, 0·05 and 0·1 M) to the *in vitro* assay containing microsomes from control animals.

A representative experiment is illustrated in Fig. 2. Animals were sacrificed at 1-, 2- and 4-day intervals of the drug regimen and at 1, 2 and 4 days post-drug treatment. The RNase activity progressively decreased to about 50 per cent of control levels as long as drug treatment was continued, and returned toward control levels 4 days after cessation of drug administration. None of the values from saline-treated

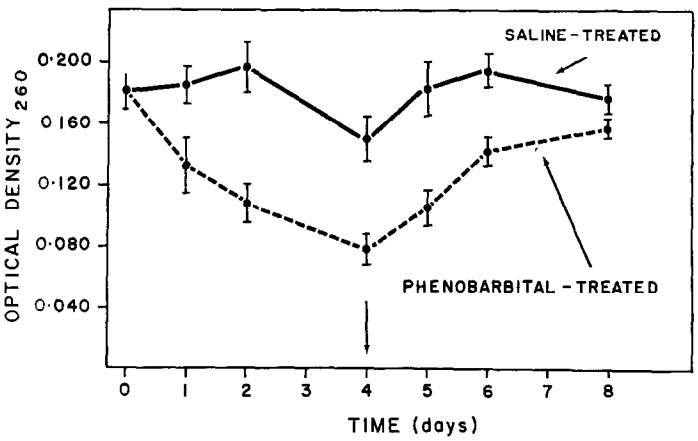

FIG. 2. Effect of phenobarbital treatment on ribonuclease activity in hepatic microsomal fractions. Animals received 10 mg/100 g of phenobarbital each day for either 1, 2 or 4 days (arrow indicates last dose). Days 5, 6 and 8 are post-drug treatment point. Units on ordinate are $O.D._{260}$/mg microsomal protein under the assay conditions described in Materials and Methods.

animals were significantly different from those of animals without treatment (day zero), while those at 2 and 4 days of drug treatment and at 1 and 2 days post-treatment differed significantly from both the saline and untreated controls ($P < 0{\cdot}001$). In a separate study, the microsomal RNase activity of 7-day phenobarbital-treated animals was compared to that of saline-treated controls; the inhibition rose to over 70 per cent.

This observed depression in RNA breakdown was closely paralleled by the development of tolerance to the hypnotic effects of barbiturates (Fig. 3). The enzymatic activity data are the same as those in Fig. 2, but are expressed as the difference of the optical density values (i.e. saline minus phenobarbital). Simultaneously with and parallel to these experiments, other animals identically treated were tested for their sleep response to hexobarbital. These data are also plotted as the difference between the sleeping time of the saline-treated rats and that of the phenobarbital-treated animals.

In another series of experiments, the induction of the oxidative drug-metabolizing activity, as determined by the *O*-demethylation of *p*-nitroanisole, was found to follow closely the inhibition of RNase activity (Fig. 4). Thus, maximal enzymatic activity (5-fold stimulation) was observed at the same time as maximal inhibition of nucleic acid degradation.

Preliminary experiments designed to determine the specificity of this type of depression in nuclease activity led to the testing of other drugs known to affect the levels of the hepatic drug-metabolizing system. The polycyclic hydrocarbon, 3-methylcholanthrene (2·5 mg/100 g, i.p.), was administered over a period of 1–4 days

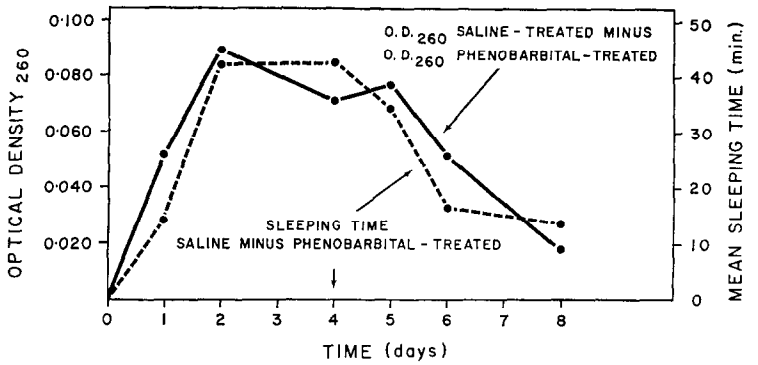

FIG. 3. Comparison of appearance of tolerance to barbiturates to inhibition of ribonuclease activity. Data for enzymatic activity (●—●) are the same as those in Fig. 2, but are expressed as the difference of the optical density values (saline minus phenobarbital). The sleep time results (●- - -●) are also expressed as the difference in minutes of the control minus the drug-treated rats.

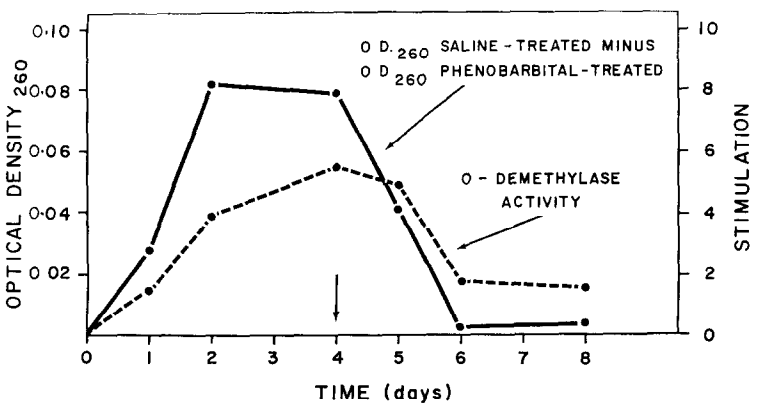

FIG. 4. Comparison of the stimulation of *O*-demethylase activity to the inhibition of ribonuclease activity. *O*-demethylase was determined as described in Materials and Methods; the data are expressed as "fold" of increase. Ribonuclease activity is expressed as in Fig. 3.

and the liver microsomal fraction was assayed for nucleic acid breakdown. At no timepoint was inhibition of nucleic acid catabolism observed.

Source of the 260 mμ *absorbing acid-soluble material*

Since the acid-soluble material formed in the course of these reactions displays its maximum absorption at 260 mμ, it might be presumed to be a derivative of nucleic acid catabolism. It was necessary to establish whether this material arises as a consequence of nucleic acid breakdown or through a synthetic process. Thus, the nucleic acid was labeled by the intraperitoneal administration of 6-^{14}C-orotic acid. As in the previous experiments, the animals were killed after 4 days of drug treatment and liver homogenates were prepared and separated into the cellular components. The radioactive microsomal fractions were then tested for RNase activity both spectrophotometrically and by subjecting the same acid-soluble filtrates for the spectrophotometric assay to radioactive determination. As the results of two separate experiments (Table 3)

TABLE 3. RELEASE OF ^{14}C FROM LIVER MICROSOMES OF RATS TREATED WITH SALINE OR PHENOBARBITAL FOR 4 days

Expt.	treatment*	O.D.$_{260}$/mg protein	Counts/min/mg protein	Counts/min released/ mg protein	Released (%)
1	None	0·145	2835	776	28·8
	Saline	0·146	2824	782	29·5
	Phenobarbital	0·048	1991	286	15·6
2	None	0·166	1439	516	36·6
	Saline	0·136	1903	608	29·9
	Phenobarbital	0·072	720	108	16·2

* The animals received a single injection of 5 μc 6-^{14}C-orotic acid on the first day, 2 hr prior to subsequent treatment.

indicate, label was released into the acid-soluble fraction in the course of the assay. A rough parallel exists between the appearance of O.D.$_{260}$ absorbing material and radioactivity. Thus, the inhibition of the RNase activity as measured spectrophotometrically was 67 and 47 per cent, while the inhibition of the amount of label released was 46 and 47 per cent respectively.

An experiment in which the release of radioactivity was studied as a function of microsomal protein is depicted in Figs. 5 and 6. It can readily be seen that the percentage of label released from the microsomal fraction of the phenobarbital-treated rats was considerably lower (13 per cent compared to 30 per cent) than that observed for saline and untreated controls and persisted over a 5-fold range of microsomal protein (Fig. 5). This inhibition was also reflected in the optical density results (Fig. 6). Although these data do not rule out the possibility that the inhibition of release

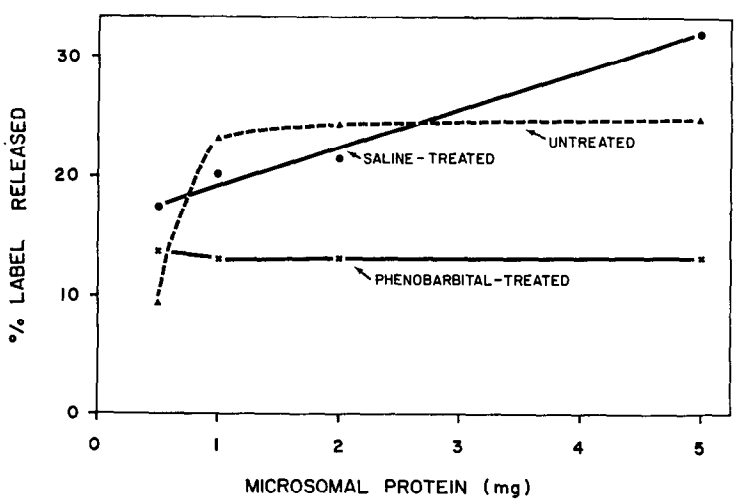

FIG. 5. The release of isotope from microsomal fractions isolated from phenobarbital-treated, saline-treated and untreated rat livers. A pulse of orotic acid-6-^{14}C (5μc/100 g) was administered 2 hr prior to subsequent administration of drug or physiological saline each day for 4 days. Animals were sacrificed on day 5. The results are expressed as the percentage of microsomal acid-soluble counts liberated during the incubation conditions found in Materials and Methods.

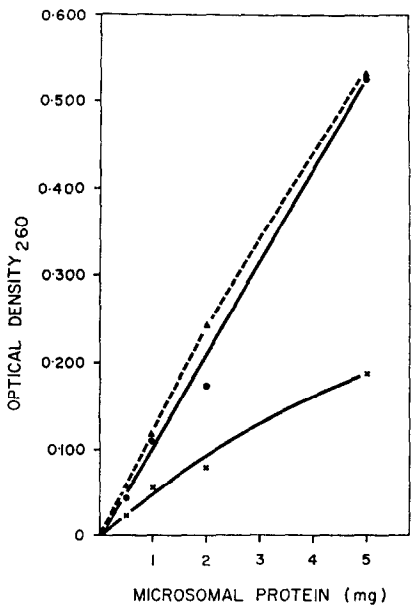

FIG. 6. Release of acid-soluble 260 mμ absorbing material from orotic acid-6-^{14}C labeled microsomes (cf. Fig. 5).

in vitro of 260 mμ absorbing material was a result of the assay conditions (that the enzyme is not completely saturated), they tend to point toward true lowering of enzymatic activity.

RNA isolated from the microsomal fractions labeled with 6-^{14}C-orotate contained radioactivity (Table 4), and thereby substantiated the view that the fragmentation of RNA was indeed being measured. Of some interest is the observation that the specific activity of the microsomal RNA from the phenobarbital-treated rats was approximately equal to that obtained from the control hepatic microsomes, while the specific activity associated with the acid-insoluble microsomal fractions of these livers did differ. On the basis of percentage of counts per gram of liver of the homogenate, the microsomal fraction derived from the drug-treated animals contained 1·5 to 3 times as much radioactivity as was found in the controls. These findings are consistent with the lower turnover of hepatic microsomal nucleic acid in the drug-treated livers. Further experiments are necessary to establish the significance of these data.

This view is further supported by an additional experiment. The enzymatic activity was determined, utilizing exogenous yeast nucleic acid as substrate (Fig. 7). Under conditions where substrate amounts were varied, the lower enzymatic activity was observed at all substrate levels. Indeed, the enzymatic activity obtained from the phenobarbital-treated animals appeared to be saturated at about half the level of the activity from control animals.

Possible role of cell sap inhibitor

As mentioned above, the presence of the cell soluble fraction in the incubation medium produced an inhibition of release of the ultraviolet-absorbing material. In

TABLE 4. RADIOACTIVITY OF MICROSOMAL FRACTIONS AND RNA DERIVED FROM LIVER HOMOGENATES OF CONTROL AND DRUG-TREATED ANIMALS 4 days AFTER 6-^{14}C-OROTIC ACID ADMINISTRATION

Expt.	Homogenate (counts/min/g liver)	Microsomes (counts/min/g liver)	(%)	RNA (counts/min/mg RNA)
Saline-treated				
1	216,756	51,243	24	49,383
2	238,793	50,976	21	51,459
3	291,497	68,966	24	51,788
Phenobarbital-treated				
1	124,887	40,181	32	52,789
2	111,760	70,614	63	47,709
3	186,261	75,591	40	99,937

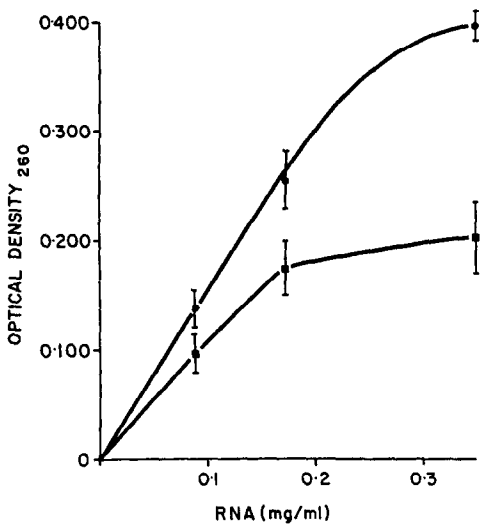

FIG. 7. Release of acid-soluble 260 mμ absorbing material employing yeast ribonucleic acid as substrate (mg/ml). Conditions of incubation are described under Materials and Methods. ●—●, Microsomal fraction from phenobarbital-treated rat liver; ■—■, microsomal fraction from saline-treated controls.

order to determine whether this was due to a greater amount of this inhibitor being present in the livers of the drug-treated animals, a crossover experiment was performed. Microsomes from saline-treated animals were resuspended either in their own cell-soluble fraction or in that from the drug-treated animals and reisolated. The same was done for the microsomes from phenobarbital-treated animals. Since no alteration of enzymatic activity was observed in the exchange experiment (Table 5), such a possibility appears unlikely.

To study further the possible role of the inhibitor, $1{\cdot}7 \times 10^{-3}$ M *p*-chloromercuribenzoate (PCMB) was added to the assay medium.[17] Roth[15] had demonstrated that sulfhydryl reactants inactivate the inhibitor and thereby disrupt the enzyme–inhibitor complex to liberate RNAase. The presence of PCMB would be expected to relieve the inhibition, if this mechanism were responsible for the lowered activity. Though its

TABLE 5. CROSSOVER EXPERIMENT

Treatment	O.D.$_{260}$/mg protein/30 min	
	Regular microsomes	After resuspension
Saline	0·169	0·142
	0·140	0·138
Phenobarbital	0·032	0·046
	0·053	0·027
% Inhibition	72·7	73·5

TABLE 6. EFFECT OF *p*-CHLOROMERCURIBENZOATE (PCMB) ON THE ENDOGENOUS RELEASE OF O.D.$_{260}$ ABSORBING ACID-SOLUBLE MATERIAL FROM MICROSOMAL FRACTION

Treatment	No. of expt.	O.D.$_{260}$/mg protein/30 min*	
		No PCMB	PCMB added
Saline	4	0·133 ± 0·007	0·211 ± 0·012
Phenobarbital	4	0·061 ± 0·009	0·135 ± 0·016

* Values are expressed as the mean ± S.E.

presence did result in a marked stimulation of release of O.D.$_{260}$ absorbing material (Table 6), the optical density of the incubations from the drug-treated animals were still much lower than those of the control animals. It is noteworthy that washing the microsomes also resulted in a small relief of the inhibition, but complete reversal was not observed. Further experiments in this area are being carried out to resolve this problem.

DISCUSSION

The results reported in this communication are in accord with the hypothesis that drug-metabolizing enzyme levels in the liver may be altered not only by a stimulation of the rate of synthesis of RNA to promote protein synthesis,[6] but also by an inhibition of the breakdown of the RNA including messenger RNA, thereby allowing for a more efficient synthesis. Data supporting the latter view also have been obtained in the laboratories of Ottolenghi *et al.*[8] and Imrie and Hutchison,[18] who studied the effect of cortisone on RNA breakdown in the liver and ACTH on RNA breakdown in the adrenal respectively. Since barbiturates interfere with the liberation of corticotrophin,[19] it is unlikely that the observed fall in hepatic ribonuclease activity due to phenobarbital administration is related to levels of adrenocortical hormones.

On the other hand, a role for the soluble ribonuclease inhibitor in depressing enzyme levels, as found in the work of RNA breakdown in the adrenal,[18] cannot be completely excluded at this time, although the crossover experiment provides evidence against its playing a role in the system reported here. The ribonuclease inhibitor of cell sap apparently plays an important part in maintaining polysomal integrity, as shown by the work of Blobel and Potter.[20] Indeed, Siler and Fried[21] in their recent study ascribe

the earlier failures to demonstrate protein synthesis using chicken liver cellular fractions to the absence of the ribonuclease inhibitor. Although the data contained in the present communication do not rule out a possible role of this inhibitor, neither do they favor it, since the crossover experiment should have detected elevated levels of this material.

The question might be raised whether the apparent inhibition is a function of the manner in which the results are expressed, since many are related to milligrams of microsomal protein. The complication arises from the fact that both an endogenous substrate and enzyme are being determined, and should (for example) the substrate not be increasing at the same rate as the microsomal protein, then an apparent inhibition might be observed. However, the results obtained with the ^{14}C-labeled microsomal fraction in which the release of label into the acid-soluble fluid is a function of microsomal protein also point towards a reduction in enzyme activity, since the percentage of label released from the phenobarbital-treated livers does not approach that released from the controls. In addition, when exogenous nucleic acid was employed as a substrate, the lower enzymatic activity persisted at all levels of substrate. The latter observation would argue against the possibility that the endogenous substrate in the drug-treated animals is more resistant to enzymatic breakdown than that from controls. However, further work would be necessary to establish this fact definitely.

An alternative explanation that might be invoked is that the ribonuclease activity is not synthesized at the same rate as are the tissue components involved with the increase in the oxygenase system. Several enzymes, including glucose 6-phosphatase and nucleoside di- and triphosphatase activities, were reported by Orrenius and Ernster[22] to be affected in this manner. More recent studies by Arias *et al.*[23] lend added support to the hypothesis that phenobarbital discriminates among proteins of the endoplasmic reticulum in promoting the synthesis of some but not of all. The mechanism for such selection remains to be elucidated. In regard to the work presented in this paper, this would essentially mean that the ribonuclease activity in the total liver has been diluted. The possible relationship to cell proliferation, such as occurs in the phenobarbital-treated liver, awaits clarification.

It is tempting to suggest that, as a consequence of the fall in ribonuclease activity, the catabolism of RNA, perhaps messenger, would be slowed and thereby permit this species to act for a longer period. A recent report indicates that no difference in turnover rates of hepatic ribosomal RNA from control and phenobarbital-treated rats is observed.[24] This seeming inconsistency with the proposed significance of the data reported herein could be resolved if the rate of turnover of the various RNA species were determined to establish whether drug treatment causes a prolongation of any one in particular.

At the present time, it is difficult to explain the observation that the inhibition occurs only in the microsomal fraction. Work on amino acid incorporation in liver microsomes and ribosomes from phenobarbital-treated rats also indicated a difference,[25] that is, more labeled amino acid is incorporated into the microsomal protein of the drug-treated animals than into that of saline controls; but when the ribosomes of these animals are employed, no difference between the two groups is observed. Although this may be taken to indicate the participation of greater amounts of messenger RNA in the microsomal fraction from the drug-treated rats, it may also imply

an involvement by the phospholipid membrane or some component associated with it. It is interesting, in this respect, that phospholipid catabolism is also reduced in phenobarbital-treated rats.[26]

Sladek and Mannering[27,82] have presented a systematic study of hepatic *N*-demethylating systems, examining the differences in the mechanisms by which polycyclic hydrocarbons and phenobarbital produce their inductive effects. Their results provide additional evidence that the polycyclic hydrocarbons stimulate drug metabolism by a mechanism different from that stimulated by phenobarbital. This conclusion is of particular interest in view of the results reported herein; that is, phenobarbital depresses the breakdown of nucleic acid in the microsomal fraction, whereas 3-methylcholanthrene has no effect. Furthermore, in considering their results, Sladek and Mannering offer the possibility that the increased activity of the hepatic drug-metabolizing enzymes might be effected by decreased catabolic processes.

Experiments are underway to establish whether a pattern exists in terms of drugs which produce inhibition of nucleic acid degradation as compared to those which have no effect. If such a pattern emerges, it would be of interest to see if it resembles that found for the cytochrome P-450–drug interaction mechanisms.[29]

After this manuscript had been prepared for publication, a communication by Louis-Ferdinand and Fuller[30] appeared. These investigators carried out similar studies, but employed exogenous substrate in all their experiments. It is of interest that our work on endogenous RNA breakdown arrives at essentially the same conclusions.

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