DISAPPEARANCE OF INDUCED ENDOPLASMIC RETICULUM AFTER CESSATION OF PHENOBARBITAL TREATMENT

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1. Introduction

The induction of the hepatic endoplasmic reticulum (ER) brought about by treatment of rats with phenobarbital has been extensively investigated (e.g. [1–5]). The activity of the drug-metabolizing cytochrome *P*-450 system increases dramatically, i.e., the process may be called substrate induction. In addition, an extensive proliferation of the ER membrane is revealed by electron microscopy (e.g. [6]) and by demonstration that the amount of microsomal phospholipid recovered per gram wet weight of liver increases 2- to 2.5-fold in response to phenobarbital (e.g. [4,7–9]).

On the other hand, very few studies on the disappearance of induced ER membranes after cessation of phenobarbital treatment have been reported. Studies which have appeared agree that the protein components NADPH-cytochrome *c* reductase and cytochrome *P*-450 return to control levels within 5–8 days after the last of five daily injections of phenobarbital [7,10]. It is not as clear what happens to the phospholipid components of the induced ER. Orrenius and Ericsson [7] found that it takes about 20 days for the amount of microsomal phospholipid per gram liver to return to control values. This finding, together with the findings for NADPH-cytochrome *c* reductase and cytochrome *P*-450, suggest that the membrane proteins are broken down, either in situ or after removal from the lipid bilayer, independently of the net breakdown of phospholipids. In contrast, a morphometric investigation carried out by Bolender and Weibel [11] with the electron microscope demonstrated that the induced ER returns to its uninduced surface area and volume within 5 days after the last injection of phenobarbital. These investigators saw an increase in the number and volume of autophagic vacuoles in rat hepatocytes during recovery from phenobarbital treatment and suggested that entire pieces of the ER – both protein and phospholipid components – are digested in these vacuoles. We have reinvestigated this problem using biochemical methods.

2. Materials and methods

Total microsomes were prepared from the livers of 180–200 g male Sprague–Dawley rats by the method of Ernster et al. [12] and rough and smooth microsomes were prepared according to Dallner [13]. Experimental animals received an intraperitoneal injection of 80 mg/kg phenobarbital in isotonic saline once daily for 5 days, while controls were injected with an equal volume of isotonic saline. Phospholipid was determined by extraction into chloroform–methanol [14] and measurement of lipid phosphorus according to Bartlett [15]. Protein was measured using a modification of the procedure of Lowry and his coworkers [16] with bovine serum albumin as standard.

Glucose-6-phosphatase [17], AMPase [18], cytochrome oxidase [19], acid phosphatase [20], NADPH-cytochrome *c* reductase [13], NADH-cytochrome *c* reductase [21], and aminopyrine demethylation [22] were assayed by reported procedures. Controls demonstrated that the latter three enzymes are not affected by CsCl in the concentrations used for the isolation of rough and smooth microsomes. When making measurements with homogenates, sonication

was employed to reveal the total AMPase and cytochrome oxidase activities. Detergent was used routinely when measuring glucose-6-phosphatase and acid phosphatase.

3. Results

Investigations involving comparison of the amount of phospholipid recovered in the total microsomal fraction from the livers of control and phenobarbital-treated rats are valid only if the recovery of ER fragments in these microsomal fractions is the same and only if the contamination of these fractions by other organelles is the same. That these important conditions are indeed fulfilled is shown by the control experiment given in table 1. No significant differences in the recoveries of marker enzymes for the ER, the inner mitochondrial membrane, lysosomes, or the plasma membrane were seen.

Figures 1 and 2 illustrate the decreases in microsomal NADPH-cytochrome *c* reductase and cytochrome *P*-450-catalyzed aminopyrine demethylation activities, respectively, after cessation of phenobarbital treatment. In agreement with other reports [7,10] these activities were found to return essentially to control values within 6 days after the last injection of the drug (fig.1A and 2A). Figures 1A and 2A also demonstrate that most of the increase in NADPH-cytochrome *c* reductase and aminopyrine demethylation is localized in smooth ER, a finding in agreement with numerous reports that phenobarbital induces proliferation of the smooth but not the rough ER (e.g. [2,3,5–7,11]). (The sum of the activities in rough and smooth microsomes was not equal to the total activity because preparation of these subfractions by centrifugation on a discontinuous sucrose gradient containing CsCl also yields an intermediate layer which is a mixture of rough and smooth microsomes. In these experiments, the more induced the animal was, the more microsomes were recovered in the intermediate layer.) However, figures 1B and 2B illustrate that the increases in the specific activities of NADPH-cytochrome *c* reductase and of aminopyrine demethylation in the rough ER are significant.

Figure 3 shows the time course for the decrease in total microsomal phospholipid after cessation of phenobarbital treatment. The maximally induced levels of total, rough, and smooth microsomal phospholipid were 207%, 113%, and 236%, respectively, of the control values. Again, this finding is in agreement with reports that phenobarbital induces the smooth but not the rough ER (see above). Total microsomal phospholipid returned from the maximally induced level to essentially control values within 6 days, a decrease which is approximately parallel to that observed with NADPH-cytochrome *c* reductase and aminopyrine demethylation. A similar

Table 1
Recovery of various marker enzymes in the total microsomal fractions from the livers of control and phenobarbital-treated rats

Enzyme	Organelle for which it is taken to be a marker	Number of expts.	% of total homogenate activity[a] recovered in total microsomal fraction from livers of: Control rats	Phenobarbital-treated rats
Glucose-6-phosphatase	Endoplasmic reticulum	4	40.3 ± 9.4	30.6 ± 5.6
Cytochrome oxidase	Inner mitochondrial membrane	3	1.39 ± 0.19	1.11 ± 0.25
Acid phosphatase	Lysosome	3	17.2 ± 0.3	20.4 ± 5.6
AMPase	Plasma membrane	3	14.9 ± 2.9	18.6 ± 2.5

[a] The figures given are means ± average deviations.

Fig.1A

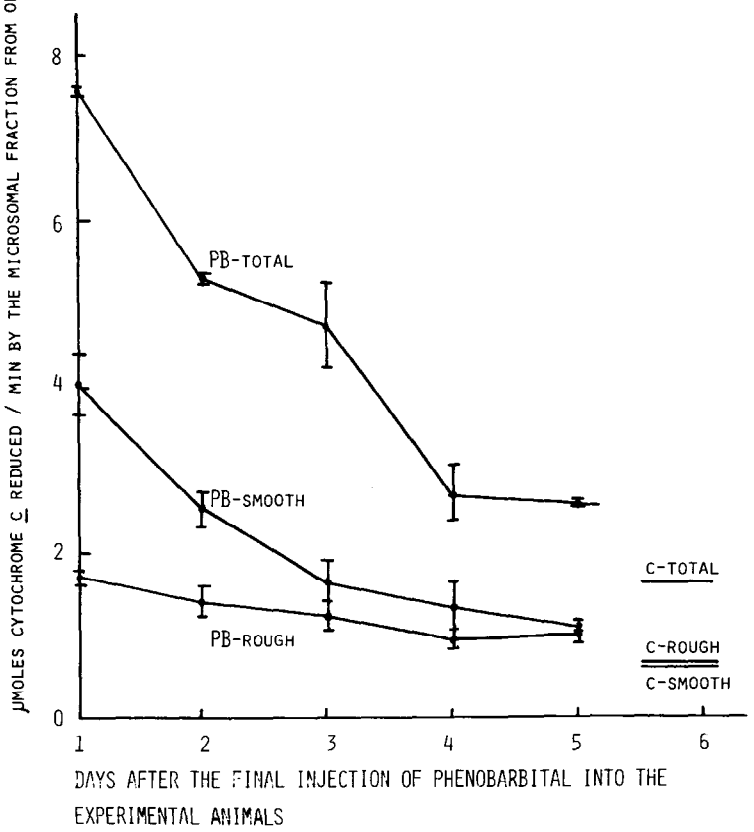

Fig.1B

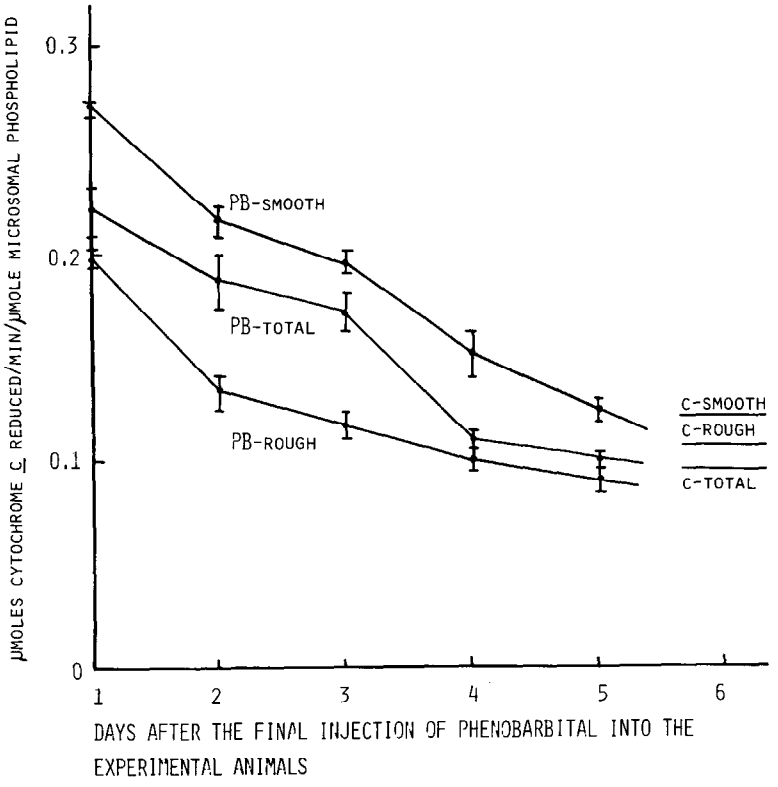

Fig.1. The decrease in microsomal NADPH-cytochrome *c* reductase activity after cessation of phenobarbital treatment. Curve A: Total activity in the microsomal fraction from one g (wet weight) of liver (this activity must be corrected for the recovery shown in table 1 to obtain the total activity per g liver). Curve B: Specific activity. PB = Microsomes from phenobarbital-treated animals. C = Microsomes from untreated animals (activities were determined on days 1 and 6 and the average of these determinations is represented by the line). The points and bars on the graph represent the means and average deviations, respectively, of three experiments.

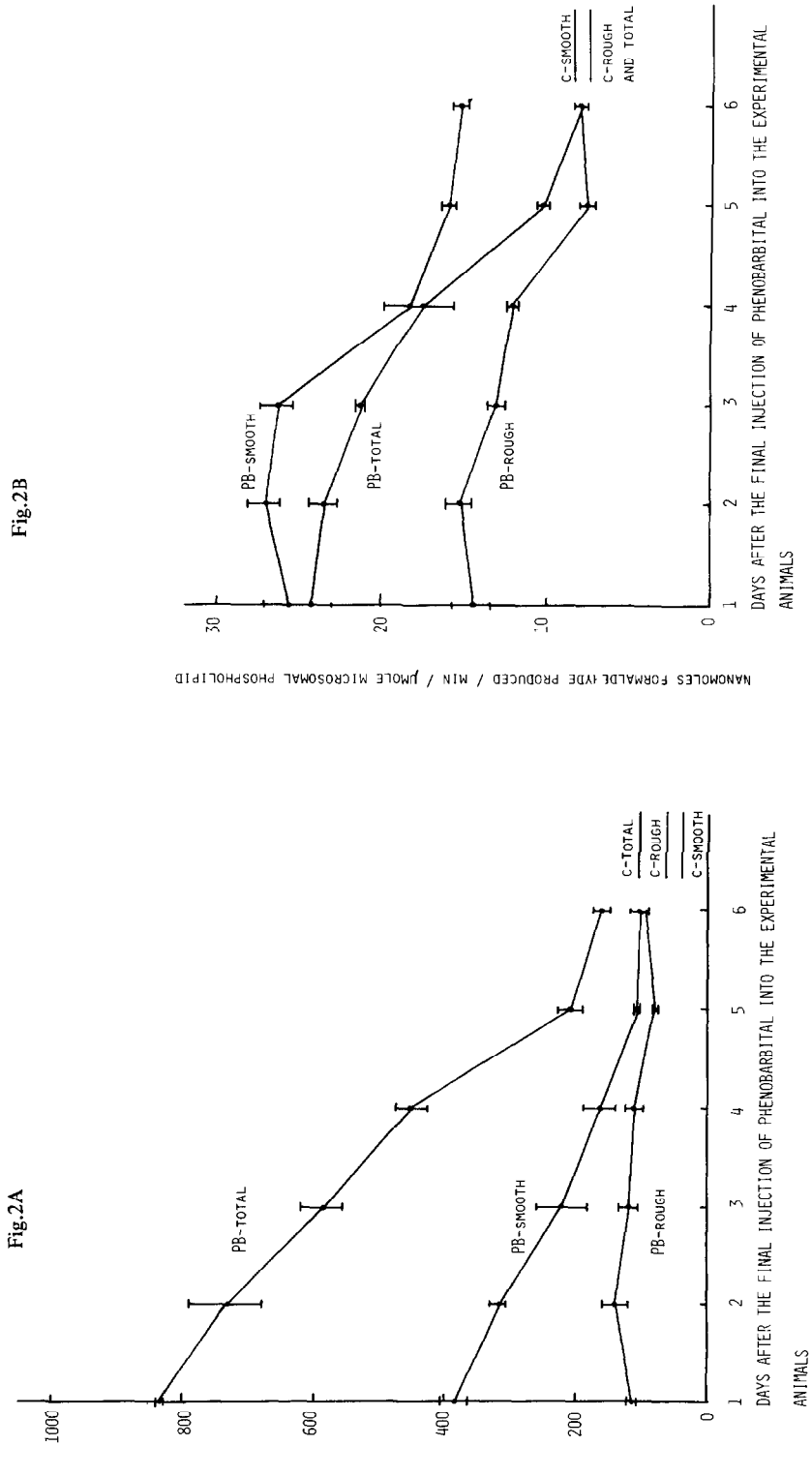

Fig. 2. The decrease in microsomal aminopyrine demethylation activity after cessation of phenobarbital treatment. See legend to fig. 1.

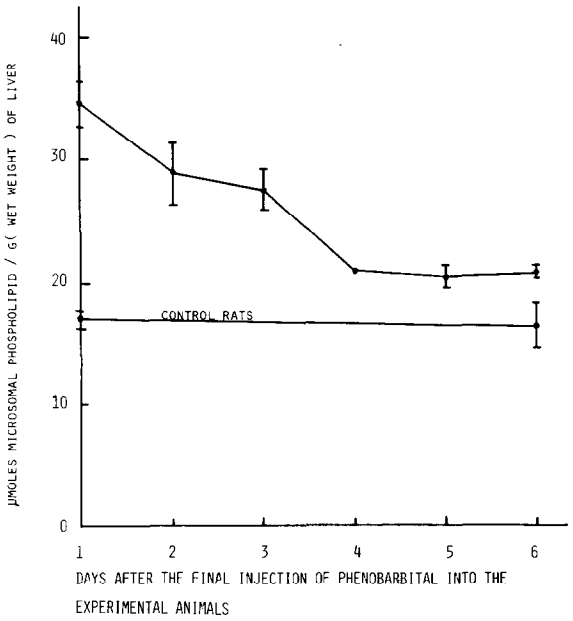

Fig.3. The decrease in microsomal phospholipid after cessation of phenobarbital treatment. The points and bars on the graph represent the means and average deviations, respectively, of three experiments.

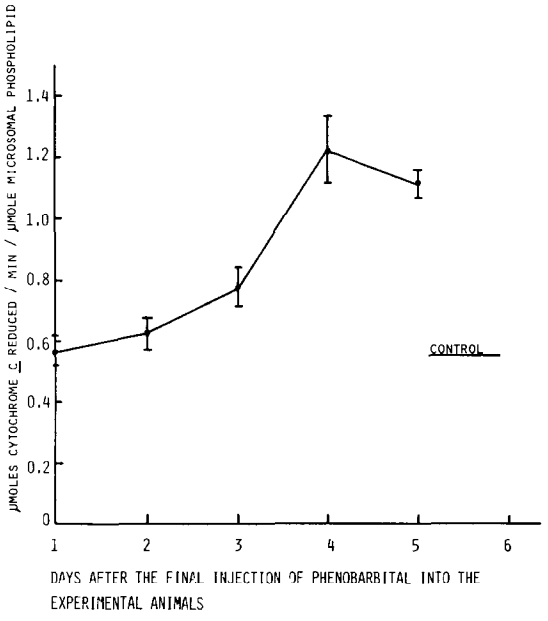

Fig.4. Change in the specific activity of microsomal NADH-cytochrome *c* reductase after cessation of phenobarbital treatment. The points and bars on the graph represent the means and average deviations, respectively, of three experiments.

pattern was seen for the decrease in microsomal protein after cessation of phenobarbital treatment. However, in this case the pattern was not as clear, probably due to the fact that phenobarbital increases the level of microsomal protein only about 40% and that a significant amount of the protein measured in a microsomal fraction is adsorbed and trapped protein which does not truly belong to the ER membrane [23].

Finally, in figure 4 the specific activity of NADH-cytochrome *c* reductase in total microsomes during the period immediately following cessation of drug treatment is illustrated. This activity is catalyzed by the microsomal enzymes NADH-cytochrome b_5 reductase and cytochrome b_5 together [24] and is not induced by phenobarbital (e.g. [17]). The total amount of NADH-cytochrome *c* reductase per gram (wet weight) of liver remained unchanged while the induced ER membranes were disappearing; but since the amount of microsomal phospholipid fell by about 50% (figure 3), the specific activity of NADH-cytochrome *c* reductase increased about 100%.

4. Discussion

It has been shown that the increased amount of phospholipid found in the microsomal fraction from the liver of phenobarbital-treated rats is not due to an increased recovery of ER fragments in this fraction or to an increased contamination of this fraction by other organelles. Rather, this increase would appear to accurately reflect the induction of the ER caused by administration of phenobarbital.

NADPH-cytochrome *c* reductase, aminopyrine demethylation, and microsomal phospholipid were all found to return almost to control levels in a parallel fashion within 6 days after cessation of phenobarbital treatment. Thus, our findings indicate concomitant degradation of the protein and phospholipid components of the induced ER. One way in which this might be accomplished is, as suggested by Bolender and Weibel [11], that entire pieces of the ER are engulfed and digested by autophagic vacuoles. However, the return of smooth and of rough ER to normal after cessation of drug treatment may involve

different processes: both protein and phospholipid components of the smooth ER are induced by phenobarbital, whereas only protein components of the rough ER are increased.

In addition, the specific activity of microsomal NADH-cytochrome *c* reductase actually increases during the disappearance of induced ER membranes. To explain this observation, one can propose either that areas of the ER with low levels of this activity are selectively degraded; or that the rates of synthesis of NADH-cytochrome b_5 reductase and cytochrome b_5 are increased to more than compensate for their rates of degradation.

Finally, it remains to be seen whether removal of the induced ER after cessation of phenobarbital treatment is a specialized process or may be a good model for the process by which cells remove damaged or non-functional organelles such as secondary lysosomes or aged mitochondria.

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