Drug-Induced Changes in the Liver Endoplasmic Reticulum: Association with Drug-Metabolizing Enzymes

Abstract. *The cellular changes which lead to the increase of drug-metabolizing enzymes following drug administration are explained by correlating new biochemical data with previously reported electron microscopic and pharmacologic observations. Repeated administration of phenobarbital and several other drugs results in a quantitative increase of the smooth endoplasmic reticulum of the liver cell. The marked increase in drug-metabolizing enzymes is found to occur in this enlarged smooth membrane fraction of the endoplasmic reticulum.*

The metabolism of various lipid-soluble drugs is accelerated in animals treated previously with numerous related and unrelated compounds. Several investigators have shown, both in vivo and in vitro, that there is a marked increase in microsomal drug-oxidizing enzymes of the liver 12 to 24 hours after administration of various drugs such as phenobarbital, nikethamide, tolbutamide, phenylbutazone, orphenadrine, and meprobamate (*1, 2*). This increase in enzyme activity may be interpreted as a nonspecific type of adaptation by the liver to various lipid-soluble drugs. A similar activation of these enzymes occurs after application of carcinogenic aromatic hydrocarbons to rats (*3*). Conney *et al.* (*2, 3*) postulated that various drugs and carcinogenic agents induce the synthesis of new drug-metabolizing enzymes, because ethionine could prevent the stimulation of enzyme activity without inhibiting the normal rate of drug oxidation. Although it appears probable that the drugs induce synthesis of new enzymes, the evidence in support of this concept has been incomplete.

Administration of one or several intraperitoneal injections of 50 to 100 mg of phenobarbital per kilogram daily or on alternate days to normally fed rats, rabbits, and dogs produced the following striking changes in the endoplasmic reticulum of liver cells examined under the electron microscope (*4*). The cytoplasm was filled with new smooth membranes, whereas numerous particles, detected by staining with lead hydroxide and believed to represent glycogen, disappeared. However, the liver glycogen content of these animals did not decrease. The increase in smooth membranes was visible 48 hours after the first injection and achieved a maximum after the fifth injection. The rough membranes of the endoplasmic reticulum, which are associated with ribosomal particles, appeared to remain unchanged. A similar increase in smooth membranes was observed by Porter and Bruni after treating rats with the carcinogen 3′-methyl-4-dimethyl aminoazobenzene; however, the appearance of new smooth membranes was followed by disorganization of rough membranes (*5*).

The microsomal fraction of the liver which contains drug-metabolizing enzymes is composed of membrane fragments of the endoplasmic reticulum and associated particles (*6*). Smooth and rough endoplasmic reticulum membranes were separated from liver cell homogenates according to the procedure of Fouts (*7*) for biochemical analysis. Electron microscopic examination of smooth and rough membrane fractions showed that separation was incomplete with a 10 to 20 percent contamination by the other fraction.

Livers of rabbits receiving one or two injections of 50 mg/kg phenobarbital showed an increase in activity of drug oxidizing enzymes, but no increase in quantity of smooth endoplasmic reticulum membranes or procaine esterase activity. Table 1 shows that smooth membranes isolated from livers of young or adult rabbits receiving six or eight intraperitoneal injections of phenobarbital (50 mg/kg) on alternate days had a very large increase in nitrogen and phosphorus content without a corresponding increase in the isolated rough membranes. No increase of nitrogen was found in other cell fractions of treated rabbits. In five rabbits treated with phenobarbital, the nitrogen content of the fraction containing nuclei and mitochondria was

Table 1. The phosphorus, nitrogen, and cytochrome b_5 content of the smooth and rough membrane fractions obtained from 1 g of liver, and enzyme activities, expressed as $m\mu$ moles of substrate, metabolized in 1 minute at 37°C, in the rough and smooth membrane fractions from 1 g liver. Hexobarbital (*11*), eunarcon (*12*), monomethylaminoantipyrine (MAAP) (*11*), and chloramphenicol (*10*) were incubated with NADP (0.4 mg), nicotinamide (12 mg), glucose-6-phosphate (10 mg), $MgSO_4$ (3 mg), the rough or smooth membrane fraction, and 1 ml liver cytoplasm from untreated rabbits containing no microsomes in a volume of 5 ml. $NADPH_2$ (*11*), procaine (*9*), glucose-6-phosphate (*14*), NADP (*15*), and ATP (*14*) were incubated with rough or smooth membrane fraction in a volume of 3 ml. The results are expressed as averages ± standard deviation.

Substance	No. of expts.	Rough membrane fraction			Smooth membrane fraction		
		Control animals	Phenobarbital-treated animals	Ratio of values for treated to control	Control animals	Phenobarbital-treated animals	Ratio of values for treated to control
P (μg)	8	202 ± 51	258 ± 57	1.39	275 ± 109	634 ± 176	2.30
N (mg)	8	1.24 ± 0.43	1.61 ± 0.57	1.30	1.58 ± 0.40	3.34 ± 0.88	2.11
Cytochrome b_5 (μmole)	6	4.3 ± 1.6	6.6 ± 3.2	1.53	8.2 ± 3.8	24.6 ± 4.5	3.0
Substrates							
$NADPH_2$	5	16 ± 7	41 ± 25	2.56	42 ± 18	155 ± 54	3.7
Hexobarbital	3	6.0 ± 2.3	10.7 ± 6.0	1.78	9.3 ± 4.3	24.0 ± 16.0	2.58
Eunarcon	3	3.3 ± 3.0	6.6 ± 3.0	2.0	3.3 ± 1.0	13.0 ± 3.0	3.95
MAAP	5	14 ± 8	21 ± 7	1.15	22 ± 7	43 ± 13	1.95
Chloramphenicol	5	0.17 ± 0.05	0.61 ± 0.34	3.6	0.24 ± 0.05	1.44 ± 0.47	6.0
Procaine	6	58 ± 24	143 ± 51	2.46	85 ± 38	345 ± 84	4.06
Enzymes							
Glucose-6-phosphatase	5	934 ± 130	1071 ± 285	1.15	608 ± 155	1054 ± 346	1.73
NADP-nucleosidase	6	59 ± 27	68 ± 17	1.15	70 ± 14	92 ± 21	1.31
ATPase	3	598 ± 110	398 ± 100	0.67	850 ± 380	820 ± 260	0.965

Table 2. Composition of the rough and smooth membrane fractions obtained from 1 g of liver from five phenobarbital-treated rabbits and five untreated rabbits, determined according to the method of Fouts (*7*). The results are expressed as averages ± standard deviation.

Substance	Rough membrane fraction			Smooth membrane fraction		
	Control animals	Phenobarbital-treated animals	Ratio of values for treated to control	Control animals	Phenobarbital-treated animals	Ratio of values for treated to control
Protein (mg)	7.0 ± 1.2	7.8 ± 2.3	1.11	8.9 ± 2.3	16.5 ± 3.2	1.85
Lipid-P (μg)	90 ± 41	137 ± 56	1.52	106 ± 35	243 ± 124	2.30
RNA (mg)	1.30 ± 0.25	1.13 ± 0.29	0.87	0.42 ± 0.10	0.65 ± 0.15	1.55
P from acid-soluble compounds (μg)	7.5 ± 1.4	7.9 ± 1.5	1.05	13.4 ± 4.3	22.4 ± 5.5	1.67

11.98 ± 1.27 mg per gram of liver, compared with 11.55 ± 1.26 in five control rabbits. The nitrogen content of cytoplasm in the treated rabbits was 11.23 ± 0.64 mg per gram of liver, compared with 10.63 ± 0.39 in the controls.

Table 2 shows that the increase in nitrogen and phosphorus found in the smooth membrane fraction after drug treatment corresponds to the increase in protein content, phospholipid phosphorus and soluble phosphorus-containing compounds. There also was a smaller rise in the RNA content. A large increase in cytochrome b_5 (Table 1) was found in the smooth membrane fraction.

These results are in accord with the experiments of Gelboin and Sokoloff who showed that previous treatment of rats with phenobarbital stimulated the incorporation of amino acids into microsomal proteins of cell-free liver preparations (*8*).

The increase in smooth membranes (approximately twofold) was associated with an even higher increase in activities of enzymes found in smooth membranes and involved in drug metabolism (two- to sixfold). Changes in the amount of activity of the enzymes that hydrolyze procaine (*9*) reduce the nitro group of chloramphenicol (*10*), demethylate monomethylaminoantipyrine (MAAP) (*11*), and oxidize hexobarbital (*11*), eunarcon (*12*), and $NADPH_2$ (*13*) are shown in Table 1. The smaller increase in enzyme activity of rough membranes after treatment with phenobarbital must be partially due to incomplete separation of smooth and rough membranes and is probably not significant. Fouts (*7*), in a study of the distribution of drug-metabolizing enzymes between rough and smooth membrane fragments, observed much smaller relative values in the rough membranes than those that are reported here.

Activities of three enzymes found in the endoplasmic reticulum, but not involved in drug metabolism were also examined (Table 1). There was a small increase in the rate of hydrolysis of glucose-6-phosphate (*14*) as a result of drug treatment. Activities of NADP-nucleosidase (*15*) and adenosine triphosphatase (*14*) did not change. Variations in the age and strains of the rabbits may account for some of the range of values obtained in identical experiments.

The three-fold rise in the amount of cytochrome b_5, determined by direct measurement of the soret band (*16*); the parallel increase in cytochrome-*c*-reductase activity (*17*); and the increased rate of procaine hydrolysis, probably provide the best evidence for a real multiplication of enzymes.

The combined morphological and biochemical observations indicate a nonspecific adaptation to injections of a drug. Besides phenobarbital, other drugs with high lipid solubility, such as tolbutamide and nikethamide, produce the liver changes described above. The result is to decrease the duration of action and effectiveness of a number of drugs metabolized to less effective or inactive compounds (*4*). Barbiturates, but not alkaloids, belong to the group of drugs producing these changes. Tolerance, developed with repeated use of barbiturates, but not morphine and other narcotic compounds, can be explained as a result of induction of enzymes produced by the drug itself (*18*, *19*).

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Polyribosomes: Size in Normal and Polio-Infected HeLa Cells

Abstract. *HeLa cells normally contain a distribution of polysome sizes, and the largest polysomes contain over 40 ribosomes. After infection with polio virus and actinomycin-D treatment, a new class of polio-induced polysomes are found, some of which contain up to 60 ribosomes. Examinaton of these polysomes suggests a mechanism for protein synthesis with this polycistronic RNA.*

Polyribosomes or polysomes are clusters of ribosomes held together by RNA. These rather than the single ribosomes are the active units for protein synthesis in vivo (*1–3*). Polysomes were first characterized in electron-microscope studies on extracts from the rabbit reticulocyte which manufactures the single protein hemoglobin (*1*). The polysomes so obtained consisted of groups of four, five, or six ribosomes which could be seen, in shadowed preparations, as tightly clustered groups or, in stained preparations, as extended ribosomal arrays. In these initial observations, the narrow distribution of polysome size was attributed to the fact that the reticulocyte was manufacturing a single protein, since the ribosomes were thought to be held together by the messenger RNA which contains the information in nucleotide sequence necessary for synthesizing the protein.

In cells, such as the mammalian HeLa cells, which are manufacturing a large variety of proteins, sedimentation studies on a sucrose gradient (*4*) indicate a broader distribution of polysome size. Analysis of pulse-labeled RNA from the polysomes of the HeLa cell strongly suggested that the ribosomes in a polysome are simultaneously attached to a single strand of messenger RNA. This interpretation was reinforced by finding a new species of polysomes in cells infected with polio virus which have been treated with actinomycin D (*4*). This antibiotic inhibits the endogenous production of messenger RNA but does not adversely affect the production of viral RNA. These new polysomes appear larger in the sucrose-gradient analysis and they are associated with the production of polio-virus specific protein (*5*). We now present the results of an electron microscope study of the polysomes in both the normal HeLa cell and in the HeLa cell which has been infected by polio virus.