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ON THE SIGNIFICANCE OF THE HYPERTROPHY OF THE SMOOTH ENDOPLASMIC RETICULUM IN LIVER CELLS AFTER ADMINISTRATION OF DRUGS

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Summary—A large increase of the smooth endoplasmic reticulum (SER) is produced in the hepatic cell following the administration of numerous drugs, some of which also have an injurious effect on the liver, while others are not capable of producing overt hepatic lesion. Morphological modifications of the SER produced by the various drugs are practically identical. However, the significance of the phenomenon is not clear, because in some cases it is accompanied by an increase, in others by an inhibition, of microsomal metabolic activity. We think that SER hypertrophy may be produced by one and the same mechanism and that all substances capable of producing SER hypertrophy may act as inducers of microsomal enzymes, thus stimulating hepatic cell to produce both endoplasmic reticulum membranes and enzymes. However, some of these substances, or their metabolites, are inhibitors of protein synthesis. In this case synthesis of new enzyme molecules is impossible and the response of the hepatic cell to the pharmacological stimulus is limited to the formation of the SER membranes.

THE HYPERTROPHY of the smooth endoplasmic reticulum (SER) in liver cells observed after treatment with various drugs, represents such a typical, and often marked finding that it immediately attracted a great deal of attention.

However, the significance of the SER hypertrophy is still uncertain. Some investigators have interpreted it as a morphological aspect of a positive phenomenon, by which the cell actively reacts to the administration of some particular drug.[1–16] Others, instead, consider it an expression of injury or even degeneration of the liver cell.[17–23]

The number of substances described as capable of producing SER hypertrophy increases daily. However, isolated observations by individual investigators are always involved, without a general interpretation of the phenomenon having been put forward.

To date the drugs known to be capable of producing SER hypertrophy are: ethionine,[1, 17, 18, 24–26] dimethyl-,[19, 27–29] and diethylnitrosamine,[20] 2-aminofluorene,[28] flurenyldiacetamide,[30] thioacetamide,[2, 3, 31–33] *p*-dimethylaminoazobenzene,[4, 34–36] 3-4-benzopyrene,[5] *p*-dimethylcholanthrene,[5] α-naphthylisothiocyanate,[37] SKF 525 A,[38] carbon tetrachloride,[6, 21, 22, 39] chlordane,[5] DDT,[5, 40] ethanol,[41] phosphorus,[23] phenobarbital,[7–12, 42] Bax 422 Z (a thiohydantoin derivative),[13] cysteine,[14] 2-methyldiazobenzene,[15] nikethamide,[16] tolbutamide.[16]

Many of these drugs are known to have an injurious effect on the liver while, in the

case of others (for instance, phenobarbital, Bax 422 Z, cysteine, 2-methyl-diazo benzene, nikethamide), no evident hepatic pathology is seen, even at high doses.

The ultrastructural modifications produced by the above mentioned drugs at the level of the SER are practically identical (Figs. 1 and 2). Moreover, from a functional aspect, all the substances mentioned show one common characteristic: all of them are involved in metabolic reactions localized in microsomes, that is, in the cell fraction containing the SER elements.

The majority of the substances indicated above as causing SER hypertrophy are metabolized by an oxidase system requiring NADPH.[43] The latter acts selectively in the metabolism of many drugs and is localized in the microsomal fraction. Some of these substances, such as phenobarbital,[44–46] 3-methyl-cholanthrene,[47–49] 3-4-benzopyrene,[48, 49] DDT,[50–52] chlordane,[53, 54] nikethamide,[16] tolbutamide,[16] behave as "inducers"; that is, they are capable of "inducing" increased activity of the NADPH-dependent oxidase system previously mentioned. The increase in enzymatic activity is accompanied by increases in microsomal proteins,[55, 56] increases in incorporation of amino acids into these proteins,[57, 58] an increase in incorporation of P^{32} into the microsomal phospholipids,[59] and an increase in the liver weight/body weight ratio.[55, 60] The increased activity of the drug-metabolizing enzymes is also accompanied by an increase in the activity of other microsomal enzymes, belonging to other systems,[11, 61–64] and of soluble enzymes.[65] These phenomena have been considered as the functional expression of SER hypertrophy.[58, 65]

However, other substances causing SER hypertrophy do not lead to an increase in drug-metabolizing enzymes,[14, 40, 67–72] It has been observed that some of them even inhibit protein sythesis.[73–85]

It seems, therefore, that the metabolic functions differ even though the morphological pattern is analogous.

We believe that SER hypertrophy is always induced as the result of a stimulus exerted by adequate doses of specific exogenous substances which affect the microsomal enzyme system destined to metabolize them. Normally, increased metabolic activity corresponds to the SER hypertrophy. However, in those cases where SER hypertrophy is not correlated to increased enzymatic activity, the substances causing proliferation of the smooth membranes, or their metabolic products, may also exert a blocking effect on protein synthesis.

It should be pointed out that administration of a typical "inducer" such as phenobarbital—which normally produces a most marked SER hypertrophy accompanied by a marked increase in drug-metabolizing enzymes—together with a typical protein synthesis inhibitor, such as actinomycin-D (which alone does not affect the SER ultrastructure,[86, 87]) leads to an increase in enzymatic activity.[10] It must also be borne in mind that SER hypertrophy and increase of microsomal metabolic activity produced by phenobarbital are closely linked, but should not be considered as the morphological and functional expression of a single phenomenon. For instance, it has been observed that increased incorporation of ^{32}P into microsomal phospholipids (a phenomenon indicating membrane proliferation) precedes the increase of enzyme activity by several hours.[10] Endoplasmic reticulum membranes seem to be formed not through *de novo* protein synthesis, but through a process of cytoplasmic rearrangement. In fact, it has been observed that the endoplasmic reticulum increases in liver cells even *in vitro* when respiration or oxidative phosphorilation are depressed.[88] This

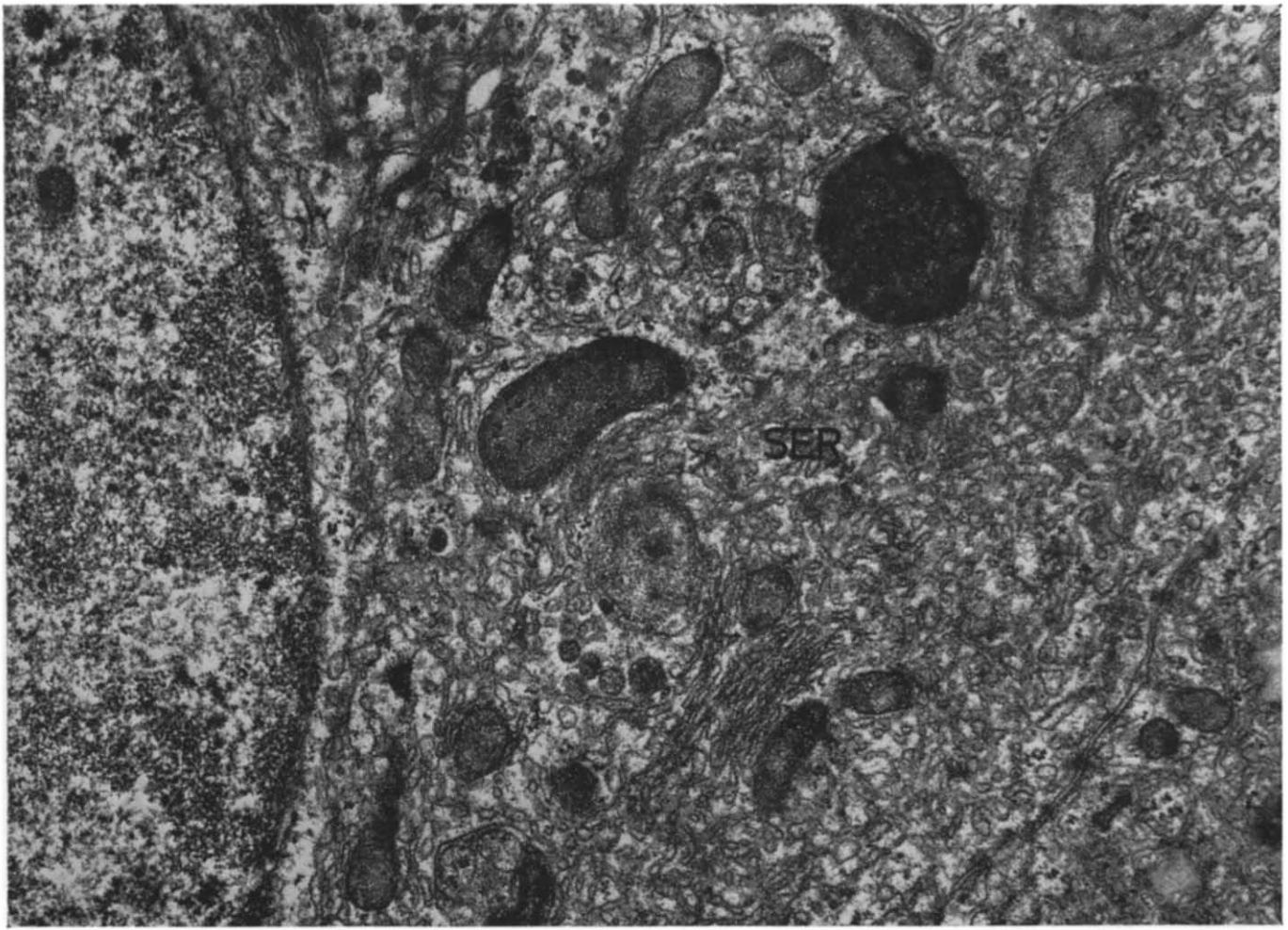

FIG. 1. Rat liver. Long term treatment with ethionine (200 mg/kg day for 10 days). Marked hypertrophy of the smooth endoplasmic reticulum (SER). Fixation—osmium tetroxide 1%. Embedding—Epon 812. Staining—uranyl acetate followed by lead citrate.

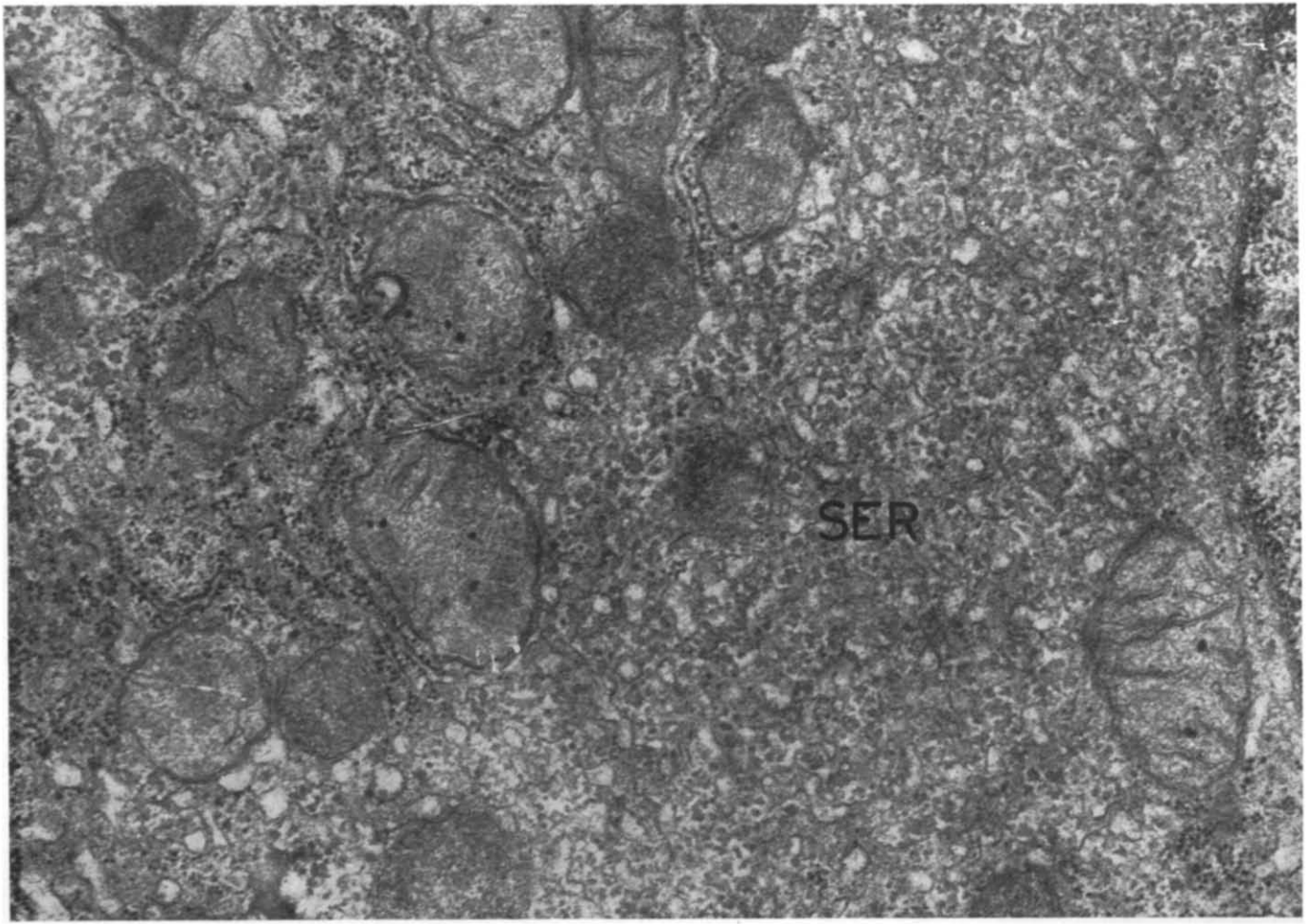

FIG. 2. Rat liver. Long term treatment with phenobarbital (5 mg/kg/day for 30 days). Marked hypertrophy of the smooth endoplasmic reticulum (SER). Fixation—glutaraldehyde 3% followed by osmium tetroxide 1%. Embedding—Vestopal W. Staining—lead acetate.

observation leads to the conclusion that endoplasmic reticulum membrane proliferation proceeds independently from metabolic activity at the ribosomal level.

The suggested hypothesis appears to hold true primarily in the case of dimethyl- and diethylnitrosamine, fluorenyldiacetamide, and aminofluorene which, like phenobarbital, are metabolized by the microsomal drug-metabolizing enzymes;[73, 74, 89, 90] their metabolites are, like actinomycin-D, powerful inhibitors of protein synthesis.[73–77]

It is possible that something similar occurs in the case of thioacetamide[78, 79, 91] and also of carbon tetrachloride. It has recently been demonstrated that carbon tetrachloride is metabolized by the hepatic microsomes. The metabolites produced are responsible for the toxicity[92–94] and also strongly inhibit protein synthesis.[80–82]

Recently it has been suggested that the mechanism of liver injury in ethanol toxicity may be analogous to that observed in carbon tetrachloride toxicity.[95]

Other substances, which are typical inhibitors of drug-metabolizing enzymes, like α-naphtylisothyocianate and SKF 525 A,[67, 72] also produce SER hypertrophy. They are known to be metabolized by drug-metabolizing enzymes,[96, 97] and their inhibiting action depends on a prolonged binding of their metabolites to the microsomes.[67, 98] Under certain experimental conditions SKF 525 A also acts as an "inducer".[97] Cysteine, which was mentioned among the substances capable of causing SER hypertrophy,[14] at high dose also leads to inhibition of drug-metabolizing enzymes.[99] Nevertheless, it increases synthesis of microsomal proteins and causes an increase in the liver weight/body weight ratio and an increase in activity of some microsomal and soluble enzymes.[14, 99] These effects are very similar to those reported for the "inducer" drugs. Furthermore, in view of the great importance of hepatic microsomes in the metabolism of sulphurated amino acids,[100] we consider it probable that an inductive mechanism is involved also in this case. Similar observations may be made for ethionine: in this case the SER hypertrophy arises after a prolonged treatment with low doses. Moreover, increased RNA synthesis[101] and enzymatic activity[102, 103] in the liver is also seen when ethionine is administered in this manner.

In contrast, the mechanism through which phosphorus produces SER hypertrophy remains unknown.

Study of the literature has led us to put forward the hypothesis that SER hypertrophy in hepatic cells is always produced by one and the same mechanism. We think that all substances capable of producing hypertrophy of the SER are metabolized by microsomal enzymes, and thus might act as potential "inducers". "Inducing" stimuli always produce smooth membrane proliferation: however, a parallel increase in enzyme activity is seen only in those cases where the "inducer" drug, or the metabolites produced, do not inhibit microsomal protein synthesis, thus permitting the formation of new enzyme molecules, which is the basis of the "induction" phenomenon.[57, 58] However, the hepatic cell seems to be capable of producing large networks of endoplasmic reticulum membranes even when protein synthesis is depressed.

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