CHANGES IN FUNCTION AND STRUCTURE OF THE ENDOPLASMIC RETICULUM OF RAT LIVER CELLS AFTER ADMINISTRATION OF CYSTEINE

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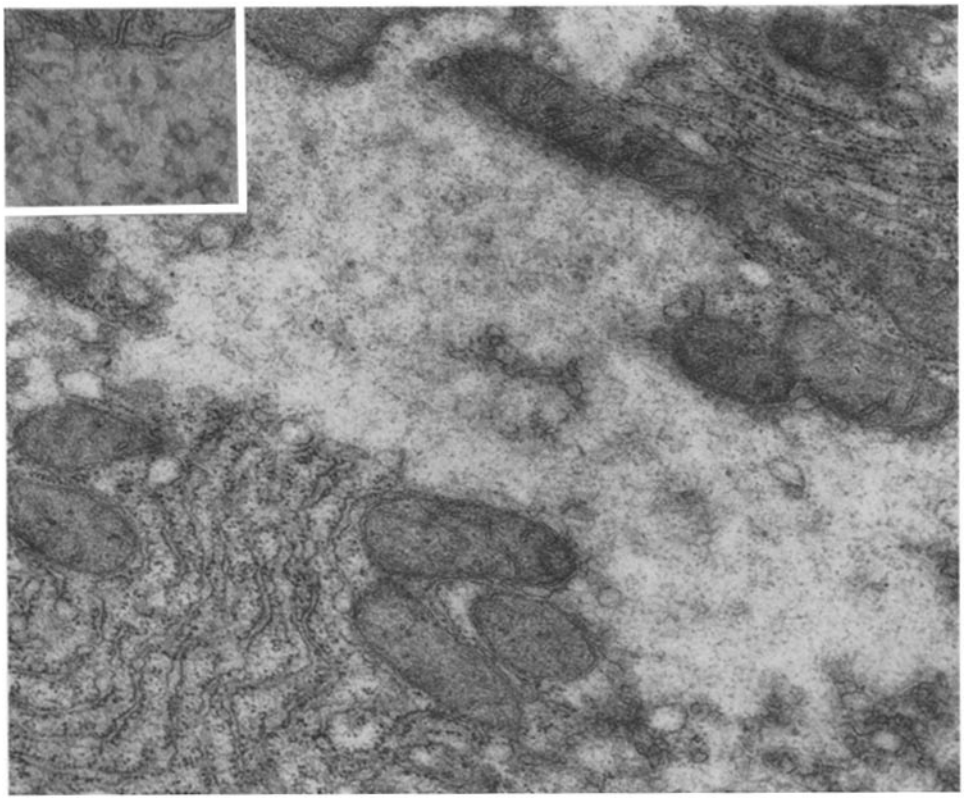
During experiments on the counteraction of the toxic effects of the carcinogen dimethylnitrosamine (1, 2) by cysteine (3, 4), it appeared that subcutaneous administration of 150 mg cysteine twice daily during 2 days to adult rats (inbred strain R-Amsterdam) led to a marked increase in the amount of material present in the liver microsomal fraction. The present paper briefly describes some of the biochemical and morphological observations pertaining to this finding of which a more comprehensive account will be given later.

All experiments were performed on the day following 2 consecutive days of cysteine administration. As a result of the latter treatment the biochemical composition of the liver was markedly altered. The RNA,[1] protein, and PL-P contents of the liver microsomal fraction (per unit volume of a suspension of the 105,000 *g* pellet prepared from a 9000 or 12,000 *g* supernatant) were increased by about 30 to 50, 35 to 70, and 25 to 50 per cent, respectively. The relative increase in the protein content of the microsomes isolated from cysteine-treated livers was consistently found to be much higher than that of the nuclear and mitochondrial fractions, which, in general, showed little change. An increase of 20 to 50 per cent in the soluble protein was also noted. The liver glycogen dropped to 60 to 90 per cent of the normal content. However, neither the concentration of s-RNA nor that of endogenous leucine present in the soluble fraction changed as a result of the cysteine treatment. Per unit amount of fresh liver weight, the DNA increased by 20 per cent; it remains to be decided whether this was due to cell shrinkage or to an increased DNA content per cell. The water content of the cysteine-treated livers was not decreased, but in most cases the liver weight (per 100 grams of body weight) was increased as compared with that of normal rats.

Incubation of the postmitochondrial fractions (9000 or 12,000 *g* supernatants, consisting of microsomes *plus* cell sap (5)) from normal and cysteine-treated livers with leucine-1-C^{14} showed a higher incorporation of label into the proteins of the latter than of the former preparations, the counts/min/mg protein being 1.5 to 2.5 times higher. Calculation showed that the cysteine-treated liver preparations incorporated from 1.4 to 2.3 as much leucine-1-C^{14} into their protein *per mg of microsomal RNA* as the corresponding system from normal rat liver. Since it was found that the binding of amino acid to the s-RNA was unchanged (3, 4), the stimulation of amino acid incorporation observed after cysteine treatment was exerted at the microsomal level. Addition of cysteine to the microsomal-soluble fraction obtained from normal rat liver had little, if any, stimulating effect on amino acid incorporation.

The effect of cysteine administration on the activities of a number of microsomal membrane-bound enzymes was also measured. The glucose-6-phosphatase was unchanged but the oxygen- and TPNH-dependent enzyme which oxidizes the *N*-methyl group of MAB to formaldehyde (*N*-demethylase) was decreased (approximately 35 per cent) in activity per unit volume of fraction (microsomes and postmitochondrial fraction, respectively) after cysteine administration (assay: 6, 7). In view of the increase in microsomal protein, the specific activity of both enzymes was decreased after cysteine administration, that of the *N*-demethylase to a greater extent (approximately

[1] Abbreviations used: RNP = ribonucleoprotein; ER = endoplasmic reticulum; RNA = ribonucleic acid; s-RNA = soluble RNA; PL-P = phospholipid phosphor; MAB = 4-monomethylaminoazobenzene; DPNH and TPNH = reduced di- and triphosphopyridine nucleotides.



FIGURES 1 TO 4

Liver sections of adult male rats of the inbred strain R-Amsterdam.

FIGURE 1

Normal liver, $KMnO_4$-stained. Parallel arrays of rough ER. In the center a large area of glycogen deposition, containing some elements of smooth ER. × 21,000. Insert: higher magnification to show glycogen units which are interconnected with, or at least closely approximate, a faint membranous network. A part of a mitochondrion at the top. × 60,500.

60 per cent) than that of the glucose-6-phosphatase (approximately 40 per cent). By contrast, the DPNH- and TPNH-cytochrome *c* reductases (6) were found to be increased per unit volume of microsomal fraction after cysteine administration in about the same proportion as the protein content of this fraction. Accordingly, the specific activities of these enzymes remained constant.

Electron microscopical examination showed definite changes in the ER of the liver cells of the cysteine-treated rats as compared to that of normal liver cells. The membrane profiles of the rough ER in the latter cells appear long and thin and in parallel arrays, few "free" RNP particles are present and smooth ER is only scarcely present in the glycogen areas (Fig. 1). Next to cells containing the usual rough ER in apparently normal amounts, other cells of the cysteine livers contained very abundant, well organized rough ER which was sometimes swollen, whereas at other places the rough ER was fragmented, showing a less parallel arrangement than that usually observed. An increase in the number of vesicular and tubular ER profiles of the smooth type was observed in the glycogen areas of the cysteine-treated liver cells (Figs. 2 and 3). Huge clusters of RNP particles, either appearing "free"[2] or attached to the surface of the small and irregularly orientated vesicles and

[2] These particles are probably attached to membranes lying within and parallel to the plane of section.

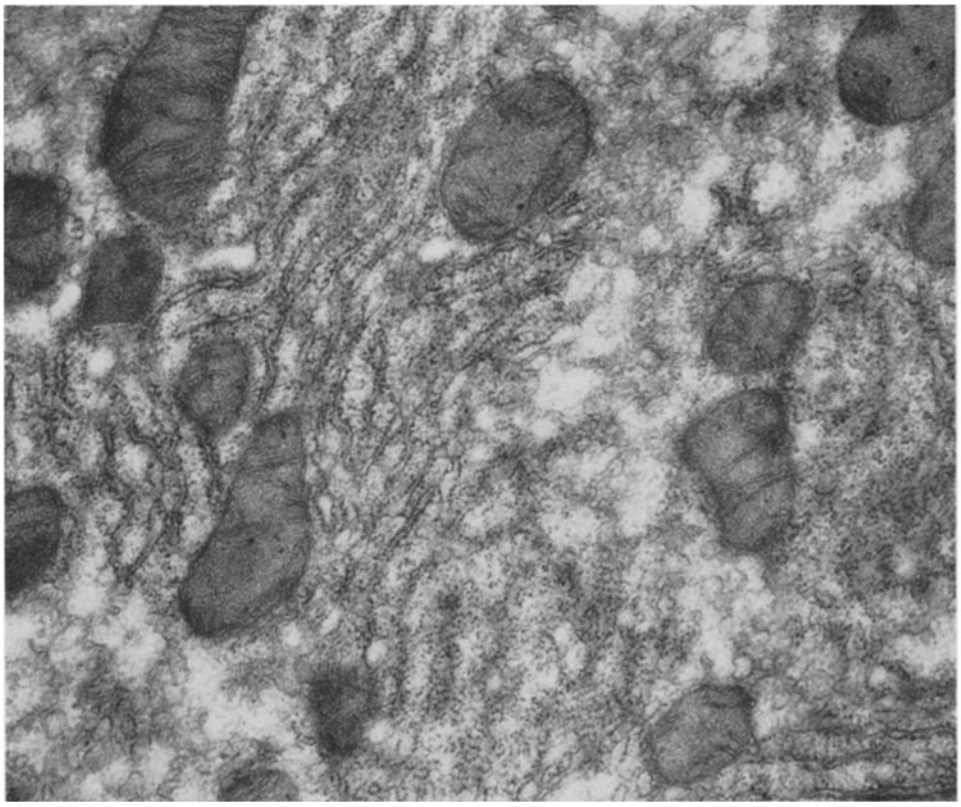


FIGURE 2

Cysteine-treated liver, $KMnO_4$-stained. Parallel arrays of rough ER (upper left). The glycogen areas contain many elements of smooth ER (vesicles and tubules) and two huge clusters of RNP particles (bottom and right). × 28,000.

tubules, or to that of a faint membranous network, were also observed in many of the cysteine-treated liver cells (Figs. 2 to 4).

The results reported in this paper show that cysteine treatment affects both the fine structure and the function of the ER of liver cells. The formation of smooth ER may in some way or another be connected with the mobilization of glycogen, as has also been suggested by observations on changes under various other conditions including fasting (2, 8, 9). The whorls and rosettes of the RNP particles may represent a stage in the formation of the rough ER as present in normal liver (10). These particles are apparently formed *de novo* and the increased amino acid incorporation observed in the cysteine-treated liver preparations per mg of microsomal RNA might be due to the activity of these particles. The reasons underlying the different responses of the various microsomal membrane-bound enzymes are not clear. It is, however, of interest that addition of cysteine to normal liver preparations has also been found (4) to lead to a marked drop in formaldehyde production from MAB.

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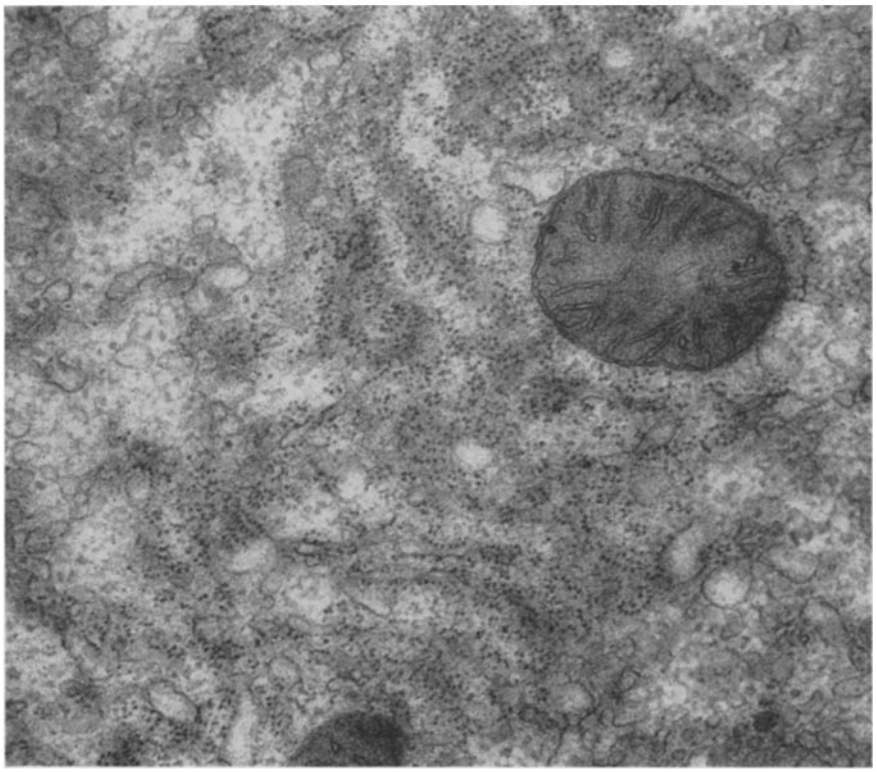

FIGURE 3

Cysteine-treated liver, stained with lead, according to Watson. Many RNP particles. Glycogen areas, at upper left and bottom right, with typical glycogen units and many smooth surfaced ER vesicles and tubules. × 37,500.

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FIGURE 4 (*on facing page*)

Cysteine-treated liver, $KMnO_4$-stained. High magnification to show the attachment of the RNP particles to a faint membranous network resulting in a thread- and rosette-like arrangement of the particles. An occasional glycogen unit of less density than that of the RNP particles may be observed (arrows). These units are also connected with faint membranes (compare insert of Fig. 1) which at several sites (*e.g.* single arrow and in the center of the figure, not indicated) appear to be connected with the membranes supporting the RNP particles. × 60,000.

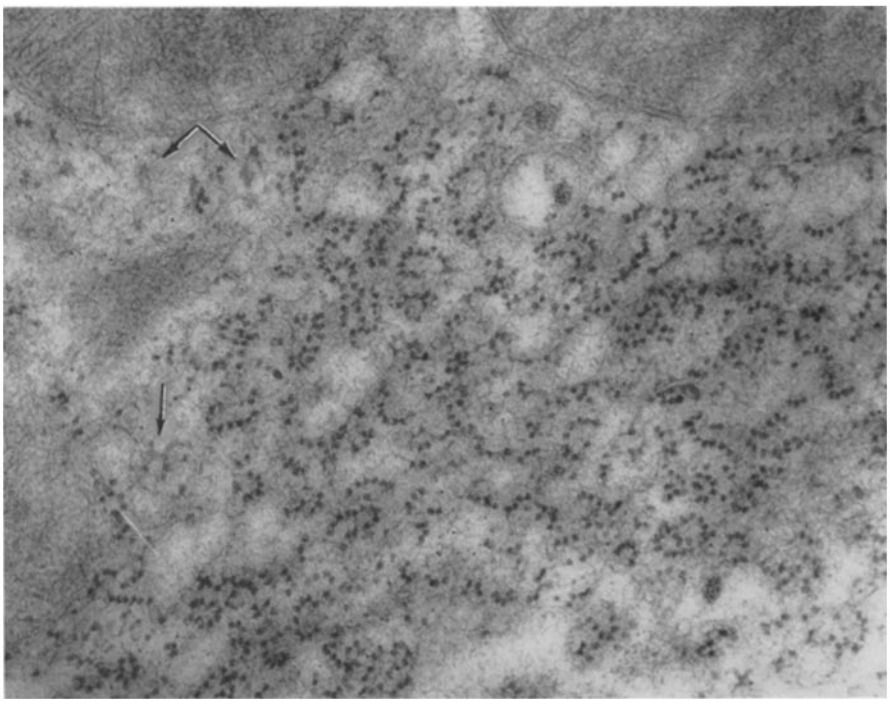

FIGURE 4