The results of this investigation thus confirm the validity of views on a possible role of immune mechanisms in the genesis of disturbances of lipid and lipoprotein metabolism. During aging, conditions favoring the greater development of autoimmune reactions and an increase in the frequency of hyperlipoproteinemia arise, and this correlates with the known increase in the severity of manifestations of atherosclerosis in the blood vessels with age.

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DISULFIDE REDUCTASE ACTIVITY IN THE REGENERATING MOUSE LIVER

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Changes in the concentrations of thiol groups [6] and cyclic nucleotides have been demonstrated in dividing liver cells. The study of the activity and control of disulfide reductase enzymes during division is important. Great interest is attached to cCMP, whose regulatory effects are almost completely unstudied.

The aim of the present investigation was to study the total disulfide reductase activity (DSRA) of enzymes capable of reducing the artificial substrate 5,5'-dithio-bis-2'-nitrobenzoate in subcellular fractions of regenerating liver. In parallel experiments activity of this individual enzyme glutathione reductase (GR) was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 129 (CBA × C57BL)F_1 mice of both sexes weighing 18-22 g and aged 3-5 months. Under superficial ether anesthesia two thirds of the weight of the liver was removed from the animals of the experimental group [7]. Laparotomy was performed on the control animals. The nuclear fraction was obtained by centrifugation of homogenates at 900g for 10 min and mitochondria by centrifugation at 9000g for 20 min. DSRA was determined by the method in [2], GR activity as in [11], and protein by Lowry's method.

EXPERIMENTAL RESULTS

DSRA and GR activity of the homogenates and mitochondrial fractions showed no significant change at the times tested. Changes in DSRA also were not found in the nuclear fraction (Table 1). Phasic changes in DSRA were found in the cytosol fraction. A small decrease in this parameter was observed in the experimental group 14 h after the operation compared with the control (by 18%). DSRA of the experimental samples increased appreciably (by 32%) after 45 h.

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These changes in activity of the disulfide reductase enzymes corresponded approximately to fluctuations in the level of sulfhydryl groups in the regenerating liver [6], possibly reflecting a link between these phenomena.

The interval of 45 h coincided with maximal activity of cell division in the regenerating liver under our laboratory conditions [4]. An increase in DSRA took place, incidentally, during induction of proliferation in mouse salivary glands [1]. By contrast, however, DSRA in tumor cells was reduced [3].

Changes in DSRA under the influence of cAMP and cCMP are given in Table 2. The stimulating effect of cAMP on DSRA was already known [3], but the activating effect of cCMP was found for the first time. The sensitivity of DSRA to the two regulators differed at different stages of regeneration, and the greatest changes were found in the nuclear fraction, cAMP activated, but cCMP did not affect DSRA in the control 14 h after the operation. In the experiment the two cyclic nucleotides had an inhibitory action, depressing DSRA by 20 and 70% respectively. In preliminary experiments, conducted at different times after the operation, effects of this kind were not observed (as a rule both nucleotides potentiated DSRA by about 10%). The appearance of inhibition is evidence of profound changes in the structure of DSRA in the nuclear fraction, due to a change in the functional state either of one individual component or of the system as a whole. It is an interesting fact that this reversal of reactivity did not take place in the cytosol. The effect of cAMP and cCMP on DSRA of the cytosol fraction 45 h after the operation was virtually absent in the experimental group. In the control, however, the usual activation was observed. Possibly during maximal mitotic activity in the regenerating liver DSRA was increased by endogenous regulators, as shown by the high basal activity of the experimental samples (Table 1).

Data on changes in GR activity of the regenerating liver are given in Table 3. The first point to note is the difference in direction of the changes in the nuclear and cytosol fractions 14 h after the operation. In the nuclear fraction distinct activation (by 73%) was observed, but some degree of inhibition (by 16%) of enzyme activity in the cytosol. The increase in GR activity in the nuclear fraction may be connected with preparation of the cells for DNA replication.

Comparison of the data in Tables 1 and 3 clearly shows that the time course of DSRA and GR differs in the regenerating liver. Probably not only GR, but also other enzymes capable of exhibiting disulfide reducing activity under these experimental conditions, and in particular, thioredoxin reductase and thiol transferase, may participate in hepatocyte proliferation.

The state of the sulfhydryl groups is known to affect the rate of polymerization of tubulin — the principal protein of the microtubules of the mitotic apparatus [9]. It has been suggested that GR participates in the polymerization-depolymerization cycle of this protein [5]. The weak point of this hypothesis is that GR cannot directly reduce protein disulfides. Meanwhile the increase in DSRA activity at the peak of mitosis in the regenerating liver indicates that reduction of the disulfide bonds of tubulin by thioredoxin reductase or thiol transferase is a possibility.

On the whole, the results are evidence that disulfide reductase enzymes participate in reparative regeneration of the liver and that the increase in concentration of thiol groups in dividing cells may be connected with changes in DSRA and GR activity. Enzymes of thiol-disulfide metabolism in different compartments of proliferating cells may differ in value,

TABLE 1. Basal DSRA (in nmoles SH groups/min/mg protein) in Regenerating Liver (M ± m)

Fraction	Time after operation, h			
	14		45	
	control	experiment	control	experiment
Nuclei	2,69±0,23 (5)	2,05±0,40 (7)	2,67±0,31 (7)	1,83±0,37 (7)
Cytosol	20,0±1,5 (13)	16,50±0,91* (16)	18,5±1,1 (22)	24,30±0,93† (23)

Legend. *P < 0.05, †P < 0.01 compared with control. Here and in Tables 2 and 3, number of animals shown in parentheses.

TABLE 2. Changes in DSRA (in nmoles SH groups/min/mg protein) under the Influence of cAMP and cCMP (M ± m)

Nucleotide	Time after operation, h			
	14		45	
	control	experiment	control	experiment
cAMP				
nuclei	+0,49±0,20 (5)	−0,47±0,14 (6)	+0,82±0,37 (13)	+0,46±0,21 (10)
cytosol	+2,25±0,15 (5)	+1,82±0,61 (8)	+1,85±0,52 (10)	+1,23±0,67 (14)
cCMP				
nuclei	−0,44±0,44 (5)	−1,38±0,52 (7)	+0,60±0,27 (13)	+0,23±0.14 (11)
cytosol	+2,02±0,35 (5)	+1,81±0,62 (8)	+1,47±0,56 (10)	−0,1±1,24 (15)

Legend. cAMP and cCMP were used in a concentration of 10^{-6} M.

TABLE 3. Basal GR Activity (in nmoles oxidized NADPH/min/mg protein) of Regenerating Liver (M ± m)

Fraction	Time after operation, h			
	14		45	
	control	experiment	control	experiment
Nuclei	11,6±2,0 (4)	20,1±1,8† (5)	16,0±1,1 (7)	15,1±2,5 (7)
Cytosol	40,3±3,1 (13)	33,7±2,6* (17)	34,2±2,2 (9)	30,2±0,51 (11)

Legend. *P < 0.02, †P < 0.01 compared with the control.

for the time course of DSRA and GR differs in the nuclear and cytosol fractions. The contrast between the fall in DSRA in the presynthetic period and its rise at the height of mitotic activity of the regenerating liver is probably a result of the greater importance of DSRA in the M phase of the hepatocyte cell cycle.

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