Sulfhydryl Levels in Regenerating Rat Liver in the Presence of Nitrogen Mustard or Protein-Depletion Diet.* (30358)

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Cyclic fluctuations in tissue sulfhydryl (SH) moieties associated with mitosis have been described in sea urchin eggs, lily anthers, yeast cell walls(1), regenerating rat liver(2), and in the aster of telephase sea urchin eggs(3). The similarities found in various species indicate a rather direct tie between mitosis and sulfhydryl metabolism. To test the closeness of this relationship, nitrogen mustard or a protein-deficient diet were given to rats during liver regeneration and the liver levels of non-protein sulfhydryl (NPSH) and protein sulfhydryl (PSH), and number of mitoses were followed. Nitrogen mustard was given as a cytotoxic agent which moderately slows the process of mitosis in regenerating liver and ultimately delays the process of regeneration(4). Normal sulfhydryl metabolism was altered by a low protein diet which markedly lowers the NPSH of normal liver(5). The results reported here further substantiate the closeness of the relationship between mitosis and NPSH.

Methods and materials. The general experimental plan has been previously reported (2). NPSH and PSH were determined according to the amperometric technique of Benesch and his co-workers(6). Nitrogen mustard‡ (Methyl-bis-[beta-chloroethyl]-amine) 1.4 mg/kg was given intraperitoneally just after the partial hepatectomy was completed. Average survival time of male 200 g rats on nitrogen mustard was about 72 hours, although 2 out of 8 animals died before 48 hours. A commercially prepared protein-depletion diet containing no protein was started 2 days before hepatectomy and continued for the entire course of the experiment. Food intake was measured and was found to be satisfactory. The liver removed for the hepatectomy was used for the control values of NPSH and PSH.

Results. The results are given in Fig. 1 and Table I. The peak of mitosis in both series of experiments occurred at the same time as that of normal regenerating liver previously reported(2), though in both instances the number of mitoses was less than normal. The NPSH levels in each series increased significantly ($P<0.01$) over control values, reaching a maximum before the peak of mitosis. This is in contrast to a maximum of 17.5 μM SH/g of liver 36 hours after hepatectomy as previously reported. The PSH values tend to be diphasic and return to normal in the case of protein-depletion diet and to remain unchanged in the case of nitrogen

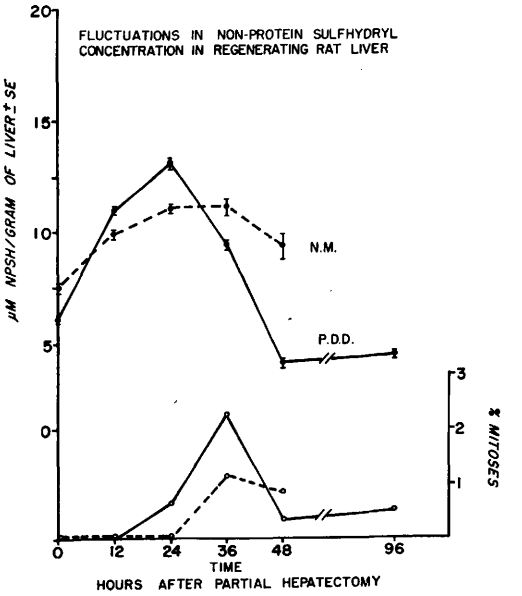

FIG. 1. Fluctuation in non-protein sulfhydryl (NPSH) levels and mitotic activity in regenerating rat liver in presence of nitrogen mustard (NM) or protein-depletion diet (PDD). Lines through the points give range of the S.E. Each point represents 8 animals except the 48-hour group of 6 animals on nitrogen mustard.

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‡ Mutagen®, Merck, Sharp & Dohme. The protein-depletion diet was obtained from Nutritional Biochemicals, Cleveland, Ohio.

TABLE I. Protein Sulfhydryl Levels in Regenerating Liver in Presence of Nitrogen Mustard or Protein-Depletion Diet.

Time*	Nitrogen mustard	Protein-depletion diet
0 (8)‡	8.8 ± .2†	7.4 ± .1
12 (8)	10.2 ± .1	10.7 ± .2
24 (8)	8.7 ± .7	6.1 ± .2
36 (8)	10.8 ± .8	5.5 ± .2
48 (6)	11.5 ± .7	8.7 ± .1
96 (8)	—	7.8 ± .1

* Time in hr after partial hepatectomy.
† Values in μM of sulfhydryl per g of liver ± S.E.
‡ No. of rats per group.

mustard. In contrast in normal regenerating liver, PSH levels drop continuously after 12 hours, showing a clear inverse relationship to NPSH.

Discussion. A decrease in liver NPSH level is a common finding under widely diversified conditions: cold restraint(7), burns and trauma tumbling(8), and protein-depletion diet(5). In contrast during liver regeneration in the presence of cytotoxic stress or altered SH metabolism the NPSH levels rise and in relation to mitotic activity. This finding is emphasized by the prompt fall in NPSH to expected low values in the protein-depletion diet after the peak in mitotic activity. The reduction in NPSH level below that of normal regenerating liver may be a result of the lesser degree of mitosis or of the effect of nitrogen mustard or protein-depletion diet to lower NPSH, or both.

The role of the increased NPSH levels prior to mitoses is uncertain and the biochemical mechanism is unknown. Two events occur which are thought to involve the sulfhydryl moieties of cell protein: (a) the formation and solation of the spindle fibers, and (b) the changes in cell wall rigidity(3,9). Both of these events involve a change in at least secondary protein structure. A rise in NPSH levels would be a potent mechanism for reducing disulfide linkages and thus conditions permitting structural rearrangement would be set up. The source and identification of the NPSH is likewise unknown. An increase in proteolytic enzyme activity which could lead to an increase in NPSH prior to mitosis has been reported(10) but such an increase was not found in normal regenerating liver(2). These findings indicate a reasonably direct influence of mitotic processes on sulfhydryl metabolism since the NPSH levels rise even in the presence of conditions which would cause them to fall if mitoses were not present. Thus the cyclic fluctuation of soluble SH compounds in mitosis seems to be more than coincidental.

Summary. The non-protein sulfhydryl (NPSH) levels of liver stimulated to regenerate by partial hepatectomy increase to a maximum about 12 hours before the maximum mitosis occurs in the presence of a low protein-depletion diet which causes a decrease in NPSH in normal liver, and in the presence of nitrogen mustard which slows the process of mitosis. This pattern is similar to that found in regenerating liver not subject to these influences. Thus a rather direct influence of the mitotic process on sulfhydryl metabolism seems to exist.

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