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Effect of Cold and Restraint on Intracellular Distribution of Glutathione in Rat Liver.* (28035)

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Beck and Linkenheimer(1) first showed that shock and cold stress decreased the nonprotein sulfhydryl (NPSH) levels in mouse liver. Bartlett and Register(2,3) reported that cold or restraint alone markedly decreased rat liver NPSH. This decrease in NPSH was shown to be primarily glutathione (GSH)(4).

The present study was undertaken to determine which intracellular fraction of the rat liver exhibited changes in GSH during stress of cold and restraint.

Methods. Female, Sprague-Dawley rats, weighing between 200 and 250 g, were employed for this experiment. These rats were previously kept on a stock diet. One-half of these animals was sacrificed and used as controls. The other half was stressed as previously described(3,5) by putting the animals in loosely fitting cages and placing in a cold room kept at 0° ± 2°C. Body temperature was so controlled that, as recorded rectally, it dropped from normal to 19-20°C over a 4 hour period. The animals were then sacrificed by decapitation and the livers were perfused with cold 0.9% saline.

The excised tissue was homogenized in 0.44 M sucrose, centrifuged at 600 *g* for 10 minutes to sediment the nuclei and cellular debris. The decanted homogenate was spun at 24,000 *g* for 15 minutes to sediment the mitochondria. The mitochondrial fraction was washed once in 0.44 M sucrose. The supernatant from the mitochondrial preparation was designated the soluble fraction. NPSH, GSH, and nitrogen was determined on the cellular fractions. NPSH was assayed by amperometric titration(8,9,4), GSH by the alloxan "305" method(4,10), and nitrogen by a standard Kjeldahl procedure.

Preliminary studies on intracellular distribution of rat liver GSH revealed that the nuclei, as prepared by the method of Chauveau *et al.*(6), or that of Hogeboom and coworkers(7), and the microsomal fraction made no significant contribution to total liver GSH. Therefore, the nuclei were removed with the cellular debris before the homogenate was used and the microsomes were not removed from the soluble fraction.

Results and discussion. Stress of cold and restraint results in a marked decrease in liver NPSH, which is principally GSH (Table I). A comparable decrease occurs in the soluble fraction with no significant change in the mitochondrial GSH. Thus the entire loss is from the soluble fraction. In stress the relative contribution of mitochondrial GSH to total liver GSH increases from 4% to 18%.

Barford and Eden(11) reported a similar intracellular distribution in rat liver with the exception of the nuclear fraction. They reported that the nuclear fraction contributed about 6% of total cellular GSH. In the present study using 2 later methods of isolating cell nuclei it was found that the nuclei contributed less than 1% of cellular GSH. No satisfactory explanation can be given for this difference.

This decrease of GSH in the soluble fraction with no change in mitochondrial fraction during cold stress was similar to the results

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TABLE I. The Intracellular Distribution of Liver NPSH and GSH in Control and Stressed Rats Maintained on Stock Diet.

Group	Assay	Units*	Homogenate	Soluble fraction	Mitochondria
16 control animals	NPSH	μmoles	3.6 ± .2	2.8 ± .2	.22 ± .01
	GSH	μmoles	3.7 ± .2	3.0 ± .2	.18 ± .01
	Nitrogen	mg	18.0 ± .6	9.9 ± .4	7.0 ± .3
16 stressed animals	NPSH	μmoles	.9 ± .1	.8 ± .1	.23 ± .01
	GSH	μmoles	1.0 ± .1	.4 ± .1	.15 ± .01
	Nitrogen	mg	17.6 ± .7	8.8 ± .5	5.9 ± .4 †

* Represent values equivalent to 1 g of rat liver. † Avg value for 10 animals.

reported by Barford and Eden(11) using dietary means of changing liver GSH concentration.

These results confirm the concept of a stable and labile fraction of GSH in the rat liver as suggested by Edwards and Westerfeld(12). Presumably about 70-80% of GSH in the soluble fraction is a labile form while the remaining 20-30% appears to be stable as is all the GSH in the mitochondria. The rat liver contains no enzyme for splitting the gamma-glutamyl linkage of GSH as reported by Binkley(13); furthermore in this laboratory no significant amount of degradation of S^{35}-GSH occurred when incubated with a liver preparation over a 4 hour period (14). It appears that the most likely mechanism for the decrease of liver GSH is increased mobilization of GSH out of the liver in excess of total synthesis as suggested by Binkley *et al.*(15).

Summary. The effect of cold stress and restraint on intracellular distribution of liver GSH was studied and compared with non-stressed rats. The decrease in the rat liver NPSH compounds, which occurs in cold stress can be accounted for by the decrease of GSH in the soluble fraction of liver tissue. The mitochondrial NPSH, most of which is GSH, does not change significantly.

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