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Functions of Glutathione in Liver and Kidney

Edited by
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Preface

The serene phrase, *Lest I forget thee, glutathione...*, coined by the Kosowers (1) to describe the state in the 1960's, must be replaced now by something like "Inevitable GSH" in order to characterize the current situation. The surge in interest on the ubiquitous tripeptide has been amazing, with publications on GSH running at rates as high as one per day, so that it seemed appropriate to convene international experts for a discussion of recent developments this year. Unlike the two previous meetings in this decade held in Tübingen in 1973 (2) and in Santa Ynez in 1975 (3), the scope was restricted to *Functions of Glutathione in Liver and Kidney*. Only in this way did an in-depth discussion of the current state of knowledge in a limited topic appear possible.

The last couple of years have seen a fascinating productivity in the fields of (a) *Regulation of the Glutathione Level in the Liver*, (b) *Role of γ-Glutamyltransferase in Glutathione Turnover* with emphasis on the renal enzyme, and a critical appraisal of the *γ-Glutamyl Cycle*, (c) *Hydroperoxide and Disulfide Metabolism*, enriched by the discovery of the nonselenium-dependent glutathione peroxidase activity and its relation to the glutathione-S-transferases, and the participation of the 2GSH/GSSG system in redox transitions in intact organ, cells and isolated mitochondria, and (d) a multitude of *Pharmacological and Toxicological Aspects* related to glutathione, mainly centered on the events leading to liver damage and the protective mechanisms. These topics were the subject matter for the meeting held as *25. Konferenz der Gesellschaft für Biologische Chemie* at Schloß Reisensburg, Germany, July 8-11, 1978.

For the sake of rapid publication of the papers containing, in most instances, brand-new information, we refrain from including the very extensive and lively discussions. Dedication and interaction of the renowned as well as the younger researchers surpassed all our expectations, and we hope that the book will transmit some of this fascination.

Autumn, 1978

HELMUT SIES
ALBRECHT WENDEL

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Contents

Chapter I
The Regulation of the Glutathione Level in the Liver

Turnover of Glutathione in Rat Liver

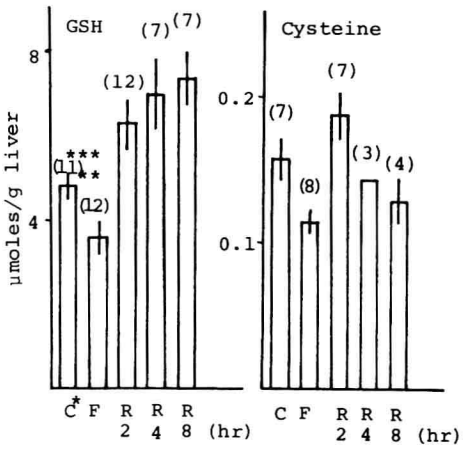
N. TATEISHI and T. HIGASHI

Summary

The glutathione content of rat liver is usually 7 to 8 μmol/g. After starving rats for one day, glutathione decreases to between two-thirds and half the normal level. When starved rats are fed a diet containing sulfur-containing amino acids, the liver glutathione level rises within a few hours. The mechanism and physiological significance of this rapid metabolic turnover of glutathione have not been elucidated. We have clearly shown that there are two pools of glutathione in rat liver, with apparently different half-lives (1.7 h and 28.5 h). The "labile" pool of glutathione functions as a reservoir of cysteine. Glutathione releases cysteine for protein synthesis when other conditions are fulfilled and the amount of cysteine becomes rate-limiting. Conversely, when excess cysteine is supplied the animals can avoid the harmful effect of excess cysteine (cystine) by storing it as liver glutathione.

A. Dependency of the Hepatic Glutathione Level on Food Intake and the Cysteine Content of the Food

When starved rats were fed a normal diet (laboratory chow), significant increases of glutathione were observed within two hours, and glutathione reached a maximum after 8 h (Fig. 1). The cysteine level in the liver also increased, but only transiently at the beginning of the feeding period (Fig. 1). The maximal level of glutathione attained depended on the amount of food ingested, with up to 10 g/day/150 g body wt. Smaller amounts of food intake always resulted in a decrease in the glutathione content, indicating a rapid turnover of glutathione (1). The quantita-



* at 6:00 p.m.
** mean ± S.D.
*** No. of rats

Fig. 1. Glutathione and cysteine contents of the livers of starved and refed rats. Male rats were fasted from 06:00 h. (C) for 24 h (starved rats = F) or fasted for the same period and then given food for 2, 4 or 8 h (refed rats = R). Glutathione was estimated by the method of Saville (4). Cysteine was determined by the method of Gaitonde (5)

tive dependency of the increase in glutathione on available cysteine was established using a protein-free diet fortified with a physiological amount of dietary cysteine. This relation can be explained by the enzymatic properties of glutathione-synthesizing enzymes and the cysteine concentration in the liver. The amounts of these enzymes did not change under the conditions we used. Of the constituent amino acids of glutathione, cysteine is probably the amino acid that limits the overall rate of glutathione synthesis, because it was always maintained at a low level (around 2×10^{-4}M) and the synthesizing system has a rather high K_m value (2.5×10^{-3}M) for cysteine (1). An increased supply of cysteine from the food should naturally increase glutathione synthesis.

B. Attenuating Effects of Other Amino Acids in the Diet (Especially L-Tryptophan), on the Increase of Liver Glutathione

Similar dependency of the increase of glutathione on cysteine intake was observed using a gelatin-diet supplemented with various amounts of cysteine (2). However, addition of protein to the diet resulted in a smaller increase of liver glutathione than that observed with protein-free diet, unless excess cysteine was supplied. This attenuating effect of gelatin was more clearly demonstrated when tryptophan was added to the diet. The increase of glutathione in rats given a gelatin-diet containing 0.18% cysteine was almost completely abolished by the further addition of 0.18% tryptophan, and only the addition of an unphysiologically large amount of cysteine was able to overcome the effect of tryptophan in suppressing an increase in glutathione.

C. Metabolic Fate of Dietary Cysteine

The effects of other amino acids, especially tryptophan, on the increase of glutathione previously described suggested that the presence of other amino acids affected the distribution of dietary cysteine in the body and altered the metabolic fate of cysteine.

For examination of the metabolic fate of cysteine derived from the food, rats were fasted for 40 h and then fed on diets containing gelatin as the protein source fortified with (1) cysteine (0.18%) (2) cysteine and tryptophan (0.18% each) or (3) excess cysteine (0.54%) and tryptophan (0.18%). L-[^{35}S]-cysteine was included in these diets. Addition of tryptophan to the diet suppressed the increase of liver glutathione. Tryptophan stimulated the incorporation of [^{35}S]-cysteine into liver and serum proteins, and diminished the amount of radioactivity recovered in liver glutathione and cysteine. When rats were given excess cysteine with tryptophan, the incorporation of [^{35}S]-cysteine into all fractions was larger. This probably indicates that under these conditions both glutathione synthesis and other systems utilizing cysteine were fully active. Addition of tryptophan to the diet induced the flow of dietary cysteine towards protein synthesis, and only the surplus cysteine was incorporated into the hepatic glutathione pool. This explanation was confirmed by additional experiments (2).

D. Transfer of Cysteine from Glutathione to Protein

The next problem was whether cysteine, once incorporated into glutathione, can be released for other purposes, in particular for protein synthesis.

Rats were maintained on an 18% gelatin diet for 4 days to reduce protein synthesis and their glutathione level. Then they were starved for one day, and given the first diet, that is, 42% gelatin diet fortified with ^{35}S-labeled cysteine, but still without tryptophan. A 42% gelatin diet was used here to give the rats sufficient gelatin in a short time. Four hours later, the rats were divided into two groups and they were given the second diet: one group was given a plain gelatin diet and the other group the same diet fortified with tryptophan. Then 11 h later, the distribution of ^{35}S in the liver and serum fractions was examined.

Within 4 h after the first diet, the liver glutathione level must have increased to 7 μmol/g or more judging from the experiments previously described. Eleven hours after the second diet, the group given a plain gelatin diet had 6.5 μmol of glutathione per g liver. However, the rats given tryptophan-containing diet had a significantly lower level of glutathione of 3.85 μmol/g liver. So tryptophan decreased the glutathione level.

Table 1 shows the distribution of ^{35}S in the liver and plasma of the two groups. The liver homogenate was similarly labeled with ^{35}S in both groups; but the intracellular distributions of radioactivity were en-

Table 1. Mobilization of cysteine moiety of liver glutathione by the administration of tryptophan

	Fortified amino acids	
The 1st diet (4 h)	Cysteine	Cysteine
The 2nd diet (11 h)	none	tryptophan
	μmol/g liver	
Glutathione	6.50	3.85
Cysteine	0.10	0.08
	x 10^{-3} cpm/g liver	
Liver		
Whole homogenate	358	357
Acid soluble fr.	197	129
Glutathione	156	91
Cysteine	3.2	2.0
Acid precipitable fr.	151	202
	x 10^{-3} cpm/ml of serum	
Serum		
Acid precipitable fr.	153	229
Albumin	58	101

Rats were given an 18% gelatin diet for 4 days. After starvation for 24 h, rats were given a 42% gelatin diet containing 0.09% cysteine and 10 μCi of L-[^{35}S] cysteine. Four hours later, rats were given 42% gelatin diet or the same amount of the diet fortified with 0.18% of tryptophan. The rats were killed 11 h after the second diet was given.