Blood and Liver Glutathione During Protein Deprivation.* (19271)

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The concentrations of both liver xanthine oxidase(1) and glutathione(2) are grossly reduced during protein deprivation. The purpose of this study was: (1) to compare the relative patterns by which these two constituents are lost from the liver during protein depletion, and (2) to see if blood glutathione reflected the liver changes so that the former might possibly serve as a criterion of protein or amino acid nutrition. Xanthine oxidase provided an index of the degree to which each liver was affected by a protein-free diet, and was therefore used as a reference point for comparative purposes.

Experimental. Adult male Sprague-Dawley rats previously maintained on Purina chow were placed on a protein-free diet for 1 to 22 days. This diet was identical with the 21% casein diet previously published(3) except that glucose replaced the casein. Groups of rats were sacrificed by decapitation at intervals and analyzed for liver xanthine oxidase (4), liver and blood glutathione. One group of rats was fed the protein-free diet to which 1% cystine was added. Reduced glutathione was determined in a sulfosalicylic acid filtrate of liver homogenate and blood by the iodometric titration of Woodward and Fry(5) after removing ascorbic acid(2). Total glutathione was determined similarly after reduction of the filtrate by the addition of Zn dust (5). Added glutathione was recovered from

TABLE I. Effect of Protein-Free Diet on Rat Blood and Liver Glutathione.

Diet			Body wt, g		Liver			Blood		
Type	Days	No. of rats	Start	End	Wt, g/100 g rat	Xanthine oxidase, $Cmm O_2/20$† min/283 mg	Reduced glutathione, mg/100 g	Total glutathione, mg/100 ml	Reduced glutathione,* mg/100 ml	Hematocrit, %
Chow	—	9	—	278	3.5	28 (24-34)	240 (167-348)	32 (20-43)	24 (18-33)	39
	1	8	237	229	3.7	20 (13-30)	134 (95-142)	29 (21-42)	27 (19-36)	39
	2	4	265	260	3.3	14 (9-19)	96 (77-109)	32 (22-40)	27 (21-33)	43
	5	4	255	238	3.6	8 (0-16)	102 (99-109)	39 (28-45)	—	41
Protein-free	6	4	284	266	3.6	5 (1-10)	84 (72-99)	45 (20-56)	—	41
	8	4	268	234	3.4	9 (0-23)	117 (86-150)	—	26 (23-30)	38
	16	4	270	215	2.7	2 (0-10)	111 (93-136)	—	33 (28-41)	33
	22	4	274	212	2.8	1 (0-2)	86 (76-95)	—	48 (40-65)	35
Protein-free + 1% cystine	6	4	298	272	3.4	9 (8-10)	442 (381-514)	—	34 (26-37)	44

Values in parentheses give the range of the results.

* The values for reduced blood glutathione are not directly comparable with the total blood glutathione since both determinations were not run simultaneously on all blood samples.

† Net oxygen consumption with hypoxanthine substrate in Cmm O_2 per 20 min per Warburg flask containing 283 mg fresh liver.

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blood and liver by this procedure with an average error of ±3 to 4% (range 100-106%).

The results are shown in Table I. Liver glutathione was reduced to nearly one-half its normal value by feeding a protein-free diet for 1 day, and it remained constant at about 40% of the starting value thereafter. Blood glutathione values were not decreased by protein deprivation; some increase was noted, first in the oxidized and then in the reduced glutathione, as the diet period progressed. This increase was not the passive result of an altered hematocrit, and may have been due to glutathione itself or to some unidentified interfering substance. Like the glutathione in normal blood(6) the reacting substance was localized within the red cell, since the plasma of rats fed a protein-free diet for 23 days was devoid of glutathione. Blood glutathione was not affected by protein depletion in such a way that its determination would be of value in assessing protein nutrition.

Feeding a protein-free diet containing 1% cystine did not prevent the loss of liver xanthine oxidase that occurred during protein deprivation, but the added cystine increased the liver glutathione markedly and the blood glutathione slightly. In a similar study Leaf and Neuberger(2) found that both cystine and methionine added to a protein-free diet restored the true liver glutathione to normal or slightly elevated values. The very high levels of liver glutathione, which they also obtained with this iodometric titration, were apparently due to a non-thiol interfering substance.

Protein depletion decreases liver xanthine oxidase and glutathione markedly, but has no large effect on these constituents in kidney, lung, muscle, or spleen(7,2). Xanthine oxidase in blood(8) and intestine(4) is also affected by a low protein diet, while glutathione is relatively unchanged. Nutritionally, liver glutathione appears to be related specifically to the sulfur-containing amino acids—possibly because it requires just one such essential amino acid for its formation.

Fig. 1 shows the comparative loss of glutathione and xanthine oxidase from the liver during protein depletion. For interpretation,

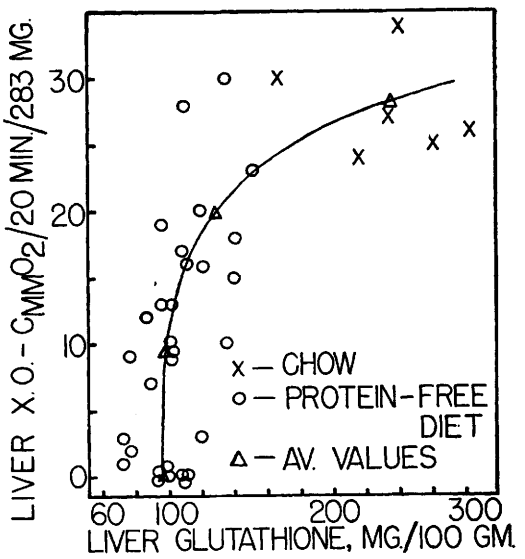

FIG. 1. Comparative loss of glutathione and xanthine oxidase from rat liver during protein depletion. Each symbol represents a liver analyzed simultaneously for reduced glutathione and xanthine oxidase.

the xanthine oxidase axis can be considered a measure of the magnitude of the protein depletion effect on the liver. Most of the labile glutathione was lost before the xanthine oxidase was seriously affected, and all of the labile glutathione was gone when the liver still retained 50% of its xanthine oxidase. Thereafter the residual liver glutathione was maintained constant while the xanthine oxidase was depleted, and when the liver contained less than 10% of its original xanthine oxidase(1), it still retained about 40% of its glutathione.

Xanthine oxidase was found quantitatively in the supernatant fraction when rat liver was homogenized in phosphate buffer and subjected to centrifugation at approximately 30000 x g for 90 minutes(1). A similar analysis for glutathione in the precipitate and supernatant fractions obtained from 4 normal and 4 protein depleted (27 days) rat livers gave the following average results:

	mg glutathione per 100 g original liver	
	Supernatant	Precipitate
Normal rat liver	180	30
Protein depleted liver	68	24

The precipitate of nuclei, mitochondria and

microsomes was not washed in these experiments and the small amounts of adhering supernatant could be expected to increase the glutathione values for the precipitates above their true levels, especially with normal rat livers. Nevertheless, the results were clear in showing that at least 85-90% of the glutathione in rat liver was present in the soluble supernatant fraction, and it was from this fraction that the labile glutathione was lost during protein depletion.

Summary. A protein-free diet reduced liver glutathione in rats to about 40% of its normal value within 1 to 2 days, but did not deplete blood glutathione. The labile 60% of the liver glutathione was lost completely before the liver xanthine oxidase was reduced more than 50%; the residual 40% of the liver glutathione was then retained while the remainder of the xanthine oxidase was lost. 85-90% of the glutathione in rat liver was present in the soluble supernatant fraction obtained by centrifuging an homogenate at 30000 x g.

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Nucleic Acid Metabolism of Bone Marrow and Spleen. I. Normal Values and Effect of Sodium Pentobarbital.* (19272)

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The nucleic acid metabolism of various organs and tumor tissue has been studied extensively(1-4) but only a few isolated observations have been reported on the intact bone marrow(5-7). This organ is of particular interest because of its very active cell division and cell maturation. We have studied the concentration and the relative rate of formation of DNA and RNA in the bone marrow and spleen of the rat. After a tracer dose of radiophosphorus (P^{32}), the DNA and RNA fractions were isolated and determinations made of the phosphorus and P^{32}. The amount of phosphorus in each fraction is a measure of the nucleic acid content, and the % of injected P^{32} per mg phosphorus, or specific activity (SA), an index of the nucleic acid newly formed during the interval studied. Thus the desoxyribose nucleic acid phosphorus (DNAP) is related to the cellularity of the bone marrow and the SA_{DNAP} an index of the rate of cell division. Further, the ribose nucleic acid phosphorus (RNAP) is related to the cytoplasmic content, while the SA_{RNAP} reflects in part the process of cell maturation. This concept is supported by experiments on irradiated rats(8), which show a close correlation between the nucleic acid determinations and the morphologic changes in the bone marrow.

In this paper, normal nucleic acid values are presented and it is shown that anesthetic doses of sodium pentobarbital produce a significant depression of DNA formation in both the bone marrow and spleen.

Methods. White male rats of the Sprague-Dawley strain, approximately 3 to 4 months old, were employed. Their weights varied

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