Effects of Subchronic Low-Protein Diet on Some Tissue Glutathione—Related Enzyme Activities in the Rat


1 Laboratoire de Toxicologie, Faculté de Pharmacie F-75006 Paris and Laboratoire de la D.A.S.E.S., F-75017 Paris, France
2 Laboratoire de Pharmacodynamie, Faculté de Pharmacie, F-34000 Montpellier, France

Introduction

Nutritional status influences the activity of drug metabolizing enzymes in the liver (Alvares et al. 1980; Basu and Dickerson 1974; Campbell and Hayes 1976; Magdalou et al. 1979). Deficiency of protein decreases the activity of the mixed-function oxidase system (cf. Hathcock 1985). Since glutathione (GS) and GS-related enzymes play an important role in the detoxification processes, the influence of subchronic low-protein diet (LPD) on GS content and GS-related enzyme activities was investigated in the liver, kidney, and gonads in the rat in order to develop an experimental system which may be used as a model to study the effect of drugs in protein-deficient animals.

Material and Methods

Animals and Experimental Procedure

Male (M) and female (F) Wistar rats, 3 week old, were fed with standard diet for 1 week. Then, 40 M and 40 F were fed with standard diet for 20 weeks (controls), 30 M and 30 F were fed for 12 weeks with an isocaloric diet containing only 5% casein (low-protein diet; LPD group), 10 M and 10 F were fed with LPD for 12 weeks and then fed with standard diet for 8 weeks (reverse group). From the control and LPD groups 10 M and 10 F were killed by carotid exsanguination after 4, 8, and 12 weeks. Remaining controls and reverse group were killed after 20 weeks. Liver, kidneys, and gonads were promptly excised and a 20% (w/v) solution of organ homogenates was prepared in 0.15 M KCl; cytosol was separated by differential sedimentation.

Analytical Methods

Total GS was determined in the cytosol using the enzymatic method of Tietze et al. (1969). The GS-related enzyme activities, peroxidase (GS Px), transferase
(GST), and reductase (GS Rd), were assayed using the method of Jaskott et al. (1983). Lipid peroxides were determined in the total homogenate by noting the thiobarbiturate reaction (Ohkawa et al. 1979). All the results were expressed relative to the proteins as measured using a modified version of Lowry’s method (Markwell 1978).

**Statistical Analysis**

All the results were statistically analyzed. The differences between the controls and the LPD rats were determined using Student’s t test and a P value of 0.05 or less was considered significant.

**Results and Discussion**

Subchronic low-protein diet (LPD) produces a spectacular decrease in body weight which is more marked in males than females. This effect on the body weight leads to an involution of the liver, kidneys, testes, and ovaries. These changes are accompanied by variations in the GS levels/mg protein and the GS-related enzyme activities/mg protein in the different tissues examined, often at the early stages of the LPD. All these abnormalities proved reversible with return to normal protein diet (Tables 1–3).

The critical role of GS in detoxification reactions is well documented. Besides its direct antioxidant role, GS serves as a cosubstrate for GS peroxidases, which reduce hydrogen peroxide and/or organic peroxides, and for GS transferases which catalyze the reaction between the nucleophilic reduced GS and the electrophilic foreign substrate, forming a less toxic GS conjugate (cf. Kaplowitz 1980 and Stadtman 1980).

In the present work a distinction must be made between various investigated tissues. In the liver and gonads of both male and female LPD-fed rats, GS was decreased as early as the 4th week. At the same time, there was a significant decrease in GS Px activity in the male and female rat liver, whereas the decrease of GS Px in the gonads of both sexes was inconstant. GST activity was significantly decreased in the male rat liver, inconstant in the female rat liver, and remained unchanged in gonads.

In the kidney, by contrast, GS and GST were increased in male and female LPD-fed rats, whereas GS Px was not significantly changed. It seems that the increased GS levels and GST activities reflect in the kidney an adaptation to the consequences of the LPD intake.

It was observed that GS Rd activity was generally increased in the various tissues of male and female LPD-fed rats, except in the female kidney and testes. GS Rd catalyzes the reduction of oxidized GS by NADPH in order to replenish the level of reduced GS that has been oxidized by free radicals, peroxides, oxygen or enzymatic pathways such as GS Px (Staal et al. 1969). This increase in GS Rd seems related to the relative increase in dextrose caloric charge due to the LPD. This load could be responsible for an increase in NADPH needed by the enzyme activity.