Effect of vitamin A deficiency on the levels of glutathione and glutathione-S-transferase activity in rat lung and liver

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Summary. Vitamin A deficiency reduces the content of glutathione in liver and lung. Also, glutathione S-transferase activity is decreased significantly in the lung, whereas its activity is increased in the liver.

Glutathione S-transferases (EC 2.5.1.18) are an important group of enzymes which catalyze the conjugation of glutathione with a wide range of compounds bearing an electrophilic center[1,2]. An inverse correlation between susceptibility to chemical carcinogenesis and glutathione S-transferase activity has been proposed[3]. Glutathione and glutathione S-transferase have been studied extensively in the liver. However, studies of their concentrations in other organs are important for the understanding of the relative susceptibility of different organs to chemical carcinogenesis.

Vitamin A deficiency has been shown to stimulate the lung carcinogenesis induced by polycyclic hydrocarbons[4], possibly by altering the activities of enzymes involved in the metabolism of these carcinogens[5]. In view of the protective role of glutathione S-transferases, it is important to study the effect of vitamin A deficiency on lung glutathione content and glutathione S-transferase activity. The present study is a part of our efforts to investigate the importance of altered activities of microsomal cytochrome P-450 dependent reactions (phase 1) and conjugation reactions (phase 2), under conditions of reduced vitamin A status, so as to assess their role in the process of chemical carcinogenesis.

Materials and Methods. Chemicals used: 1-Chloro 2,4-dinitrobenzene (CDNB) was purchased from Aldrich chemical Co., Milwaukee, WI, USA. Glutathione (GSH) and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were of reagent grade.

Male rats (50-60 g) of the Institute's colony (Wistar derived) were used. They were maintained for 5-6 weeks on a vitamin A deficient casein-based diet[6,7]. Control animals received orally 240.0 µg vitamin A acetate twice a week and were pair-fed to the deficient animals. Animals had free access to water and food during the 1st 4 weeks, thereafter the amount of diet supplied to the control animals was reduced in proportion to that consumed by the deficient group. All animals were sacrificed by exsanguination under light ether anesthesia. The lungs were perfused in situ through the right ventricle with 40-50 ml 0.15 M NaCl and the liver through the portal vein, so as to reduce the blood contamination to a minimum. Lungs and liver were quickly excised and rinsed in cold KCl/Tris buffer (150 mM-KCl/50 mM-Tris/HCl), pH 7.4. Tissues were diced with scissors, homogenized in a Potter-Elvehjem homogenizer and diluted to a concentration of approximately 1 g wet wt/2 ml with KCl/Tris buffer solution. This was centrifuged and the supernatant was used for glutathione estimation by the method of Moron et al.[8], using DTNB to give a compound which absorbs at 412 nm. For each set of assays a standard curve using GSH was prepared.

The rest of the supernatant was diluted with KCl/Tris buffer and centrifuged at 105,000 × g for 60 min in a Beckman ultracentrifuge model L5-50B. The supernatant was used for estimating glutathione S-transferase activity by the method of Habig et al.[9], using CDNB as substrate. Enzyme assays were done under conditions giving activities linear with respect to incubation times and protein concentrations. Protein was determined by the method of Lowry et al.[10], using dried BSA as standard. Liver vitamin A was estimated as described by Dugan et al.[11]. Statistical analyses were done by Student's t-test for significance at the $p < 0.05$ level.

Results and discussion. Table 1 shows that there are no differences in the body and organ weights between control and deficient animals. Vitamin A concentration in the control group was about 60 µg/g liver; it was not more than 2 µg/g liver in deficient animals. Glutathione content and glutathione S-transferase activities in the lungs and livers of the 2 groups are given in table 2. Vitamin A deficiency resulted in a significant decrease in the content of glutathione both in liver (40.7%) and lung (46.6%). In vitamin A deficient animals the activity of glutathione S-transferase was significantly increased in liver (46.3%), whereas its activity in lung was significantly decreased (41.3%).

There have been a few studies showing the effect of low body vitamin A status on the enzymes involved in the metabolism of chemical carcinogens in liver[5,7]. Relatively little attention has been paid to the altered levels of these enzymes in their target organs (in this case lung), in vitamin A deficiency. The lungs are directly exposed through inhalation to environmental pollutants like benzo(a)pyrene, a major carcinogenic constituent of smoke[12]. Chhabra et al.[13] indicated that the liver and lung glutathione S-transferase activities were not affected by inducing vitamin A deficiency in rabbits and guinea-pigs. Recently Siddik et al.[14] have reported that glutathione S-transferase activity in liver was significantly increased, whereas there

Table 1. Status of rats

	Control	Deficient	
Body weight (g)	181 ± 18(7)	177 ± 15(10)	NS
Organs weight (g)			
Liver	7.5 ± 1.5(7)	7.3 ± 1.3(10)	NS
Lung	1.58 ± 0.25(7)	1.48 ± 0.23(10)	NS
Vitamin A content (µg/g)			
Liver	60 ± 7.01(7)	1.1 ± 0.45(10)	$p < 0.001$

Results are expressed as mean ± SD of the number of determinations given in parentheses. NS, no significant difference.

Table 2. Effect of vitamin A deficiency on lung and liver glutathione content and glutathione-S-transferase activity

	Control	Deficient	
Glutathione content			
Liver	5.50 ± 0.74	3.26 ± 0.43	$p < 0.01$
Lung	1.18 ± 0.21	0.63 ± 0.08	$p < 0.01$
Glutathione-S-transferase			
Liver	574 ± 76	840 ± 89	$p < 0.01$
Lung	93.4 ± 15	54.8 ± 10	$p < 0.01$

Content of glutathione (µmoles/g of tissue) and enzyme activities (nmoles of CDNB conjugated/min/mg of protein) are expressed as mean ± SD of 6 determinations. The level of significance of the difference between control and deficient groups is given. See the text for experimental details.

was no change in the pulmonary glutathione S-transferase activity in vitamin A deficient rats. This is partly in disagreement with our findings as we have observed a significant decrease in pulmonary glutathione S-transferase activity. This could be due to strain variation. The exact mechanism, however, is not very clear. Moreover, the content of glutathione was significantly low in both liver and lung of these rats. Therefore, in addition to a decrease in the activity of glutathione S-transferase in the lung, the low content of glutathione in lung, where the normal level of glutathione is far less than that in liver, may result in prolonged failure to maintain an adequate intracellular supply of glutathione for conjugation and possibly be one of the factors towards enhanced susceptibility to lung cancer in vitamin A deficiency.

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The dietary origin of the urinary lignan HPMF

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Summary. The influence of a synthetic diet devoid of plant fiber material on the occurrence of HPMF, a mammalian lignan, in rat urine was studied. The urine of rats on normal food contained significant amounts of HPMF. When the normal food was replaced by the synthetic diet the HPMF content fell to near-zero levels.

Two new compounds present in animal and human urine have recently been identified as trans-(±)-3,4-bis[(3-hydroxyphenyl)methyl]dihydro-2-(3H)furanone (HPMF) and a closely related butane-diol[2,3]. These substances are the first examples of lignans to be found in mammals, but their origin and biological importance are still unknown. Because of the cyclic pattern of its excretion in women[4] and the female vervet monkey[5], HPMF was first thought to originate from the gonads. From a recent study, however, it was concluded that HPMF and the diol are formed by intestinal bacteria[6].

Since lignans occur exclusively in higher plants we have, as a first step towards establishing the origin of these compounds, investigated the effect of the diet on the urinary HPMF levels in the rat. We have compared the effects of a normal rat diet and a semi-synthetic diet devoid of lignan and lignin-containing plant constituents. In addition we investigated the effect of gonadectomy. In this way we aimed to establish whether HPMF is formed from lignans, lignin or related substances derived from plants, or whether its formation is independent of the diet.

Material and methods. Adult male and female rats of Wistar origin were housed individually in metabolism cages, and fed normal rat food (RMH-B, Hope Farms, Woerden, The Netherlands) for 8 days. They were then changed to a synthetic diet of the following composition: casein (22%),

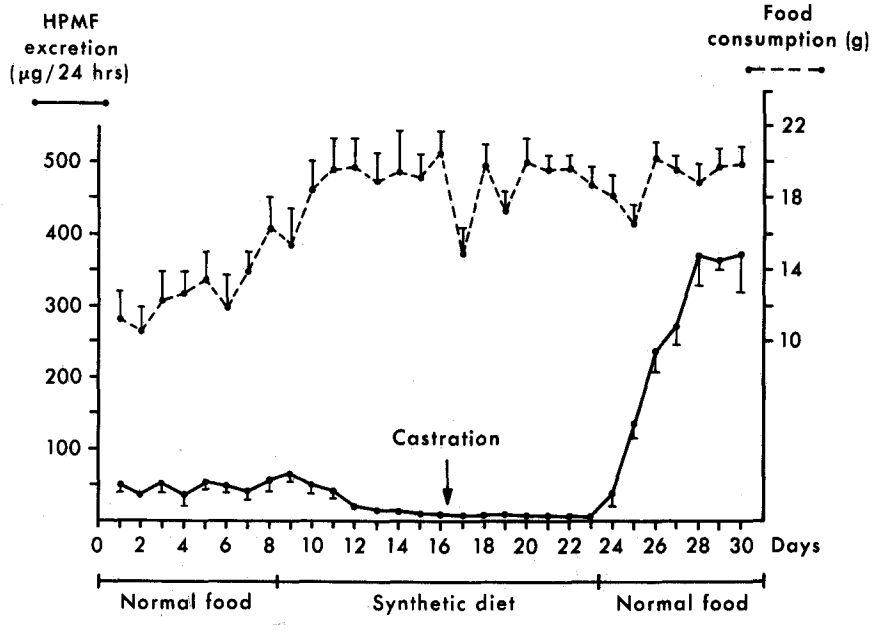

Figure 1. Food consumption and urinary HPMF excretion of male rats fed normal rat food and a synthetic diet. The values are given as means (n=6). The bars represent SEM.