The effects of vitamin A nutritional status on glutathione, glutathione transferase and glutathione peroxidase activities in rat intestine

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Summary. In rat intestine, the glutathione level was increased, glutathione peroxidase activity decreased and glutathione-S-transferase unchanged by vitamin A deficiency.
Key words. Vitamin A; glutathione; glutathione peroxidase; glutathione transferase; intestine.

Some xenobiotics may initiate cellular lipid peroxidation either directly by generating free radicals[1] or indirectly by decreasing the effectiveness of various biological defence mechanisms. Among these, glutathione, which possesses direct antioxidant functions, also contributes largely to detoxication processes by its action with various peroxidases and transferases. Glutathione peroxidase prevents cellular damage through the detoxication of hydrogen peroxide and lipid hydroperoxides[2]. Glutathione-S-transferases act in the detoxication of a wide range of compounds that have an electrophilic center subject to nucleophilic attack by glutathione[3]. An inverse correlation between susceptibility to chemical carcinogenesis and glutathione-S-transferases has been demonstrated[4]. Furthermore, Burk et al.[5] mentioned an additional factor conferring protection against lipid peroxidation which is thought to be due, at least in part, to some of the glutathione transferases.

These detoxication processes can be influenced by dietary factors such as natural antioxidants[6]. It is known that vitamin A deficiency enhances the susceptibility of the organism to chemical carcinogenesis[7], and recently Tom et al.[8] found that peroxidation of rat liver microsomal lipids in vitro was inversely related to dietary intake of vitamin A. The effects of a vitamin A deficiency on these detoxication systems have already been studied in the liver. However, formation of free radicals can occur in other organs, especially those exposed to high concentrations of xenobiotics, like the intestine. As vitamin A deficiency is known to enhance the vulnerability of the gut to carcinogens[9], we undertook this experiment to observe the effects of such a deficiency on intestinal glutathione content, glutathione transferase activity and particularly glutathione peroxidase activity, which has not yet been studied.

Materials and methods. Male weanling Sprague-Dawley rats (50–60 g) were maintained for 9 weeks on either a vitamin-A-free diet (deficient) or an identical diet supplemented with retinyl acetate (20000 IU/kg) (control). Animals had free access to water and food. Rats were killed by decapitation after having been fasted overnight. The proximal intestinal segment was flushed with saline (0.9% w/v NaCl) to remove mucus and food residues, slit open and the mucosae were scraped, at 0 °C, with the blunt edge of a glass slide. The mucosal scrapings were then sonicated in 0.25 M sucrose, 0.05 M Tris-HCl, 1 mM EDTA, pH 7.4. The intestinal sonicate was centrifuged for 20 min at 9000 × g and the supernatant fraction spun at 105,000 × g at 4 °C for 1h. Cytosolic protein concentration was assayed by the method of Lowry et al.[10] using bovine serum albumin as standard. The cytosol was used for estimating glutathione-S-transferase activity (GST) by the method of Habig et al.[11] using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. Nonprotein sulfhydryl groups (mostly reduced glutathione, GSH) were estimated by the method of Sedlack and Lindsay[12], and selenium dependent glutathione peroxidase activity ((Se)GSHPx) by the method of Burk et al.[13] using hydrogen peroxide as the substrate. Statistical comparisons were made by using analysis of variance (test F).

Results and discussion (table). After 9 weeks, body weight was significantly reduced in vitamin-A-deficient rats as compared to controls. Using the same conditions, Siddik et al.[14] showed that hepatic vitamin A levels began to decline soon after the animals were placed on a vitamin-A-free diet and that by 9 weeks hepatic vitamin A was not detectable, which could explain the growth deficit observed.

Intestinal GST activity was not altered by vitamin A deficiency, and this result contrasts with that of Nair et al.[15]. However, several glutathione-S-transferases exist, which possess overlapping specificities[16], and we did not use the same substrate to measure this enzyme activity. As in the liver, the highest specific activity is observed with CDNB[17], which we used as the substrate, whereas the activities towards other substrates like those used by Nair et al.[15] (1,2 dichloro-4-nitrobenzene and p-nitrobenzyl chloride) are much lower.

Effects of vitamin A deficiency on body weight and on intestinal cytosolic protein concentration, glutathione level, glutathione peroxidase and glutathione transferase activities

	Control	Deficient	
Body weight (g)	360 ± 5	337 ± 5	$p < 0.01$
Cytosolic protein (mg/g cell)	82.8 ± 2.3	81.3 ± 2.3	NS
Glutathione-S-transferase (nmoles/min/mg protein)	251 ± 11	262 ± 12	NS
Glutathione level (nmoles/mg protein)	14.6 ± 0.6	17.9 ± 1.0	$p < 0.01$
Selenium dependent glutathione peroxidase (nmoles NADPH/min/mg protein)	6.2 ± 0.3	4.9 ± 0.2	$p < 0.01$

Results are means ± SEM from 16 rats. The level of significance of the difference between control and deficient groups is given. NS, no significant difference. See the text for experimental details.

Our results show that vitamin A deficiency results in a significant increase in GSH content (23%) and a significant decrease in (Se)GSHPx activity (21%). These variations appeared to be specific responses to deficiency, since the total intestinal cytosol protein concentration was comparable in both groups. The increase in intestinal GSH level, as reported previously[18], contrasts with the depression of GSH levels observed in liver and lung under the same conditions[19, 20]. This rise in intestinal GSH level could indicate an adaptative mechanism by which the intestine exhibits its defence against a possible threat from the environment.

The protective role of vitamin A against carcinogenesis seems to be related to the function of the vitamin in the control of normal differentiation of epithelial tissues[7]. However, several authors have shown that vitamin A deficiency could also affect some phase I and phase II drug-metabolizing enzyme activities, depending upon animal species and tissue assayed[13,21,22], and thus modify the proportion of active and inactive metabolites of potential chemical carcinogens. Moreover Dogra et al.[23] showed that such a deficiency could modify the activities of protective enzymes against free radicals in lung and liver. In this study we found that (Se)GSHPx activity was unrelated to glutathione levels and that this biological defence mechanism was affected by vitamin A deficiency. This decrease in (Se)GSHPx activity may significantly contribute to the accumulation of peroxides and, in spite of the increase in GSH content, could be responsible, at least in part, for the greater susceptibility of vitamin A deficient intestine to chemical toxicity and carcinogenesis.

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Inactivation of sarin and soman by cyclodextrins in vitro

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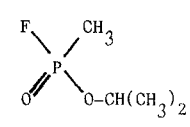
Summary. Cyclodextrins catalyzed the inactivation of sarin and soman but did not inactivate tabun and VX. Furthermore, sarin and soman showed greater affinity for β-cyclodextrin than for α- or γ-cyclodextrins. Thus β-cyclodextrin appears to be an attractive starting material for the preparation of a catalyst able to inactivate sarin and soman more effectively. Such a catalyst might contribute to improving the therapy of poisoning caused by these two nerve agents.
Key words. Nerve agents; sarin; soman; tabun; VX; cyclodextrin.

Cyclodextrins (cycloamyloses, CD) are toroidal oligosaccharides composed of α-(1,4)-linkages of a number of D(+)-glucopyranose units produced from starch by *Bacillus macerans*[1]. This report deals with the inactivating effect of α-cyclodextrin (cyclohexamylose), β-cyclodextrin (cycloheptaamylose) and γ-cyclodextrin (cyclooctaamylose) on the following potent anticholinesteratic nerve agents: sarin, soman, tabun and VX.

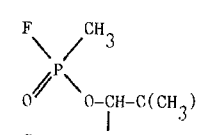
It is a well-known fact that cyclodextrins can bind large organic molecules (substrates) and then attack them within the complex through their secondary hydroxyl group (s) or through the corresponding alkoxide ion(s)[2]. Thus cyclodextrins are able to catalyze the hydrolysis of certain organophosphorus compounds: diaryl pyrophosphates[3], isopropyl 4-nitrophenyl methylphosphonate[4], diaryl methylphosphonates[5] and dimethyl 4-nitrophenylphosphate[6]. In particular, it has been shown that 1) soman is a good substrate for β-cyclodextrin at pH 7.40[7,8] 2) sarin is a rather poor substrate for α-cyclodextrin at pH 7.40[8] and 9.00[9,10] and for β-cyclodextrin under the same conditions[8] but that 3) tabun is not a substrate for γ- and β-cyclodextrin[8]. However, dissociation constants of the cyclodextrin-substrate complexes and phosphonylation constants (see below) were measured only when using α-cyclodextrin/sarin[9,10] and β-cyclodextrin/soman[7,8] systems. It appeared interesting therefore to obtain the constant values, not yet determined, which characterize the interaction between each cyclodextrin and each nerve agent considered. The aim of this determination was to find out whether one of the cyclodextrins had more affinity with these four organophosphorus compounds than the others. The cyclodextrin which most efficiently forms a complex with the nerve agents and which could, in addition, be covalently modified in order to improve its nucleophilic attack on these within the complex, might be a useful component in the therapy of poisoning by these compounds.

Material and methods. Sarin, soman, tabun and VX obtained from the Centre d'Etudes du Bouchet, Vert-le-Petit, France, were racemic compounds at least 98% pure. Stock solutions of nerve agents (2 mg/ml) in absolute ethanol were kept at −40 °C. Cyclodextrins and acetylcholinesterase (type VI-S) were purchased from Sigma, St-Louis, Mo. All other reagents were of analytical grade.

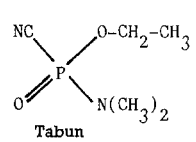
The mechanism consistent with covalent catalysis of nerve agents PX by cyclodextrins is given in the scheme of figure 1. Dissociation constant K_d and rate constants k_2 and k_3 correspond to the various processes indicated, k_o being the rate constant for spontaneous hydrolysis of the nerve agent. This mechanism is identical with the enzymatic Michaelis-Menten mechanism. If $|CD|_o$ and $|PX|_o$ are the initial concentrations of cyclo-



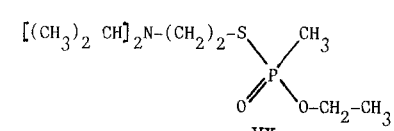
Sarin



Soman



Tabun



VX