a lack of any direct cardiotoxic effect of this drug. These observations indicate that the OH· radical, if produced via reaction I, did not require the presence of iron. Here it should be noted that the binding ratio between desferal and ferric iron is 100/8.5 parts by weight [14]. Desferal complexes with iron to form a stable chelate ferrioxamine which does not allow iron participation into chemical reactions [14]. It should be noted that this drug is freely permeable and is now in use for the treatment of iron overload [11].

A systematic approach was taken to establish which of the oxygen radicals produced in the perfusion system may have been responsible for the contractile failure. Since the X–XO combination has been suggested to produce superoxide radical anion ($O_2^{\cdot-}$) according to reaction IV, the enzyme superoxide dismutase (SOD, 30,000 and 120,000 units/l, Sigma) was added to the perfusion. Superoxide dismutase alone in the KH control solution had no effect on the functional properties of the perfused hearts (data not shown). A significant protective effect against X–XO induced contractile failure at the higher concentration of SOD (Tables 1 and 2) provides evidence that the $O_2^{\cdot-}$-radical is involved. In a recently published report, Blaustein *et al.* [15] failed to show any protection with SOD; this negative finding may have been due to the low concentrations of the enzyme used. Since at the lower dose we also did not see any protection (Tables 1 and 2), it apparently is a dose-dependent phenomenon.

To establish whether the $O_2^{\cdot-}$ radical involvement is direct or indirect through the production of other toxic oxygen species such as OH· and H_2O_2, we studied the effects of mannitol (a scavenger for OH·) and catalase (a scavenger for H_2O_2) on X–XO induced contractile failure. Both mannitol (10 and 20 mM, Sigma) and catalase (20,000 and 40,000 units/l, Sigma) had no effect on the force developed in the presence of KH control medium. Both of these agents were found to be protective against X–XO induced contractile failure (Tables 1 and 2). Since mannitol and catalase do not affect $O_2^{\cdot-}$ production, the depressant effect on this radical may be indirect, through OH· and/or H_2O_2 formation. The protection offered by SOD noted above could have been due to a reduction in the superoxide-dependent OH· radical formation. In this regard, SOD has been shown to depress the hydroxyl radical formation [8]. The decline in developed force as well as in dF/dt may be due to the peroxidation of polyunsaturated fatty acids in different subcellular components including mitochondria [2, 12]. In fact, loss of structure–function integrity of the membranes as well as high energy phosphates in rat hearts exposed to X–XO was reported earlier [12].

In conclusion, the present use of desferal as a chelator has shown that iron as a catalyst may not be an absolute requirement in the Haber–Weiss reaction for the generation of OH· radicals in the X–XO system. The observation has strong implications for the selection of an antioxidant to prevent oxygen radical induced tissue injury because iron chelation may not always be successful in *in vivo* interruption of oxygen radical production.

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St Boniface General Hospital Research Center and Department of Physiology Faculty of Medicine University of Manitoba Winnipeg, Canada R3E 0W3

MADHU GUPTA
PAWAN K. SINGAL*

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* Author to whom all correspondence should be addressed.

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Relationship between age of mice, enzymes such as acetylcholinesterase and aliesterase, and toxicity of soman (pinacolyl methylphosphonofluoridate)

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Organophosphorus anticholinesterases are generally more toxic to very young animals [1–7]. This sensitivity of young animals to the toxic effects of organophosphates could result from an underdeveloped cholinergic nervous system [1, 8, 9] and/or an immature organophosphate detoxification system [2, 5, 10, 11].

Acetylcholinesterase (EC.3.1.1.7) inhibition is the primary biochemical lesion following soman (pinacolyl methylphosphonofluoridate) poisoning. Binding to aliesterase (EC.3.1.1.1; carboxylic ester hydrolase) is an important detoxification route for soman [12]. The purpose of this study was to compare the enzyme activities of acetylcholinesterase and aliesterase, as a function of age, to the toxicity of an organophosphate anticholinesterase, soman, in mice.

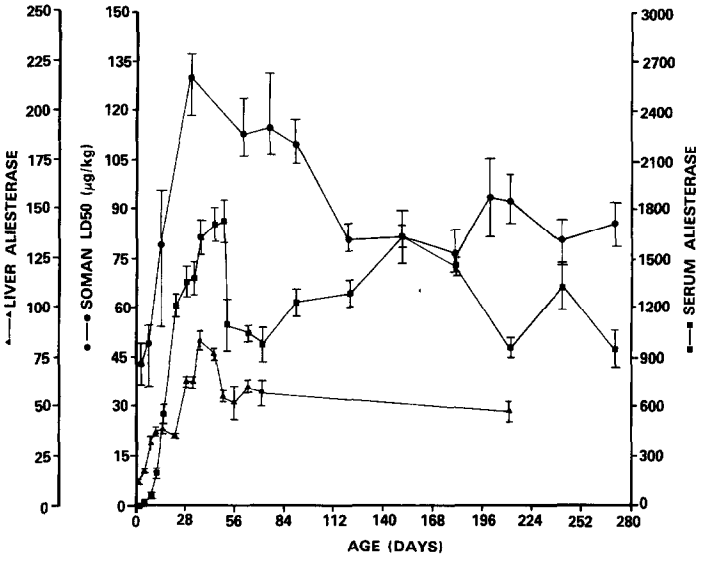

Fig. 1. Serum and liver aliesterase activity and LD_{50} of soman as a function of age in male mice. Each point represents mean ± SEM (N = 6–10). Serum aliesterase activity is expressed as nmol tributyrin hydrolysed/ml serum/min and liver aliesterase as nmol tributyrin hydrolysed/mg protein/min.

Materials and methods

Soman, >98% pure, was prepared at the Defence Research Establishment Suffield. Soman was dissolved in 0.9% saline immediately before use. The following chemicals were obtained from commercial sources: tributyrin (Sigma) and [^{14}C]acetylcholine (4 mCi/mmol; New England Nuclear).

Mice (CD-1^{R}) were obtained from Charles River Canada Ltd., St. Constant, Quebec. They were kept in our vivarium at a temperature of 23° and a photoperoid of 12 hr with lights on at 7:00 a.m. Animals were allowed access to food and water *ad lib*.

Male mice were killed by decapitation followed by exsanguination. Tissues were removed, homogenates were prepared, and acetylcholinesterase and aliesterase activities were determined according to previously described procedures [13].

In the toxicity experiments, soman dissolved in saline was injected s.c. in to male mice. Ten animals per dose and at least four different doses of soman were used in determining the LD_{50} by probit analysis [14].

Results and discussion

During the early stages of development, there was a large increase in liver and serum aliesterase activity which peaked at 36 days in liver and 36–49 days in serum (Fig. 1). By day 60, there was a sharp drop in aliesterase activity. Thereafter, the serum and liver aliesterase activity remained relatively unchanged. The soman LD_{50} value peaked around 30 days at 130 μg/kg, then decreased to 110 μg/kg by 90 days, and stabilized in the 77–94 μg/kg range over the remainder of the observation period. It appears that the peak in the soman LD_{50} is coincident with the peak in serum and liver aliesterase activity but not the acetylcholinesterase activity (Fig. 2).

In mice, there is a strong correlation between the level of serum aliesterase and soman toxicity [13]. Soman binding to "serum" aliesterase was concluded to represent the primary detoxification route following subcutaneous administration. The term detoxification is used in the context that, if soman is bound to serum aliesterase, it is not free to inhibit synaptic acetylcholinesterase. As serum aliesterase levels decrease, the toxicity of soman increases and *vice versa* [13]. However, in the present study, beyond 60 days of age, this generally did not occur. For example, there was no significant difference in serum aliesterase activity at 90 and 240 days of age (Fig. 1), yet the soman LD_{50} values were significantly ($P < 0.05$) different. This suggests that possibly the soman binding capacity of serum aliesterase changed with age.

Similar to the results found by others for various organophosphates [1–7], young mice were more susceptible to the toxic effects of soman. It is doubtful that an immature metabolic system is responsible for this observation since soman is not metabolized by the hepatic mixed-function oxidase system [15]. Phosphorylphosphatase hydrolyzes some of the four stereoisomers of soman but only the nontoxic ones [16] and, therefore, does not play a role in the *in vivo* detoxification of the toxic stereoisomers of soman [12, 16].

In summary, in the early stages of development, the toxicity of soman was related to serum aliesterase activity. The susceptibility of young mice to the toxic effects of soman was probably not due to an immature drug-metabolizing system. In older mice (day 90 and greater), the toxicity of soman did not appear to be related directly to serum aliesterase levels and may indicate that the soman binding capacity of serum aliesterase changes with age.

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Biomedical Defence Section, Defence Research Establishment Suffield Ralston, Alberta, Canada T0J 2N0

SUSAN PEET
J. DEBORAH SHILOFF
JOHN G. CLEMENT*

* To whom all correspondence should be sent.

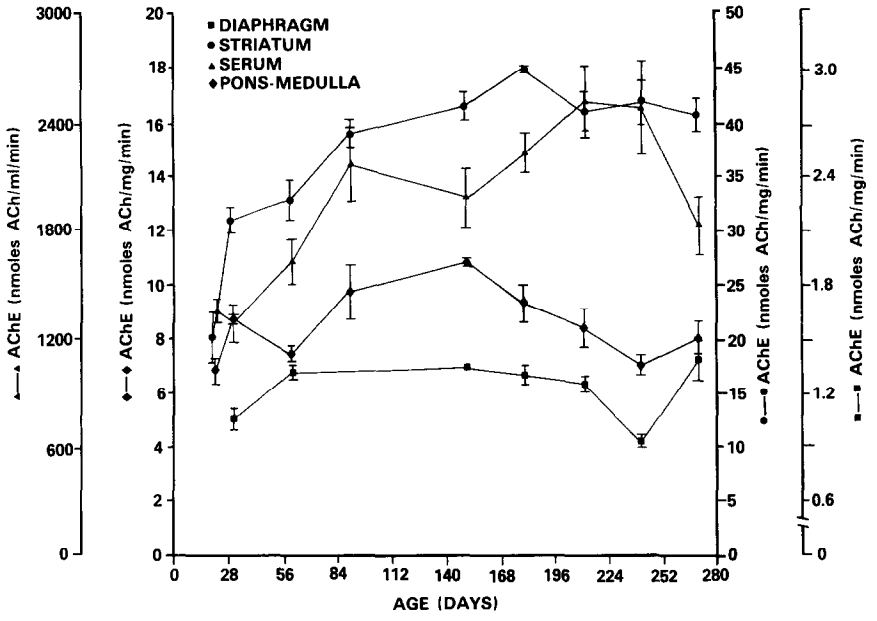

Fig. 2. Acetylcholinesterase activity in various tissues as a function of age in male mice. Each point represents mean ± SEM (N = 5).

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