Table 2. Recovery of 5-ASA and Ac-5-ASA following the administration of dietary sulfasalazine to rats and guinea pigs*

	Recovery (%)			
	Urine†	Feces		Total
	Ac-5-ASA	5-ASA	Ac-5-ASA	
Rats	23.1 ± 2.0	29.8 ± 2.4	23.9 ± 3.3	76.7 ± 5.3
Guinea pigs	36.0 ± 3.6	2.6 ± 1.0	15.8 ± 2.0	54.3 ± 4.7

* Five rats received a diet containing 1% sulfasalazine from day 1 until day 4, which gave the individual animals doses equivalent to 213, 232, 238, 269 and 279 mg of 5-ASA during the 4-day period. Six guinea pigs received a diet containing 1% sulfasalazine from day 1 until day 4, which gave the individual animals doses equivalent to 120, 129, 132, 134, 159 and 162 mg of 5-ASA during the 4-day period. Total recovery is expressed as the mean ± SD in terms of the percentage of 5-ASA contained in the administered sulfasalazine.

† 5-ASA was not detected in the urine of any animal.

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Oxygen radical injury in the presence of desferal, a specific iron-chelating agent

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Oxygen radicals and activated oxygen species, products of biological reduction of oxygen, have been implicated in the pathophysiology of tissue injury due to ischemia-reperfusion, side effects of drugs, irradiation and inflammation [1, 2]. One of the important chemical reactions for the formation of these toxic species was proposed in 1934 by Haber and Weiss [3], as shown below:

$$H_2O_2 + O_2^{\cdot-} \rightarrow OH^- + OH^{\cdot} + O_2 \quad (I)$$

This reaction is thermodynamically feasible and has been documented as a source of the highly toxic hydroxyl radical ($OH^{\cdot}$) [4–6]. However, under chemically well-defined conditions, the reaction has been suggested to be kinetically very slow or even negligible [7, 8]. the overall stoichiometry of the often cited Haber–Weiss reaction is widely accepted, but the reaction has been suggested to be a combination of the following two half-reactions requiring iron as the catalyst as described below [8]:

$$O_2^{\cdot-} + Fe^{3+} - EDTA \rightarrow O_2 + Fe^{2+} - EDTA \quad (II)$$

$$Fe^{2+} - EDTA + H_2O_2 \rightarrow Fe^{3+} - EDTA + OH^- + OH^{\cdot} \quad (III)$$

$$H_2O_2 + O_2^{\cdot-} \rightarrow OH^- + OH^{\cdot} + O_2 \quad (I)$$

Requirement of iron in reactions II and III for the production of $OH^{\cdot}$ (reaction I) has also been demonstrated in *in vitro* hydroxylation studies [9]. Hydroxylation of aromatic compounds with xanthine–xanthine oxidase, at pH 7.4, could not occur until low concentrations (μM range) of $FeSO_4$ or $FeCl_3$ were provided in the medium [9]. The

combination of purine and xanthine oxidase in the perfusion medium was found to cause a significant depression of contractile function and myocardial cell damage in isolated myocardial septal preparations only upon the addition of 2.4 μM Fe^{3+} loaded transferrin into the perfusion system [10]. This observation further supported the importance of iron in the production of oxygen radicals via reaction I. In the present study on isolated perfused rat hearts we show that xanthine–xanthine oxidase (X–XO) induced depression of contractile function and this effect can occur in the presence of Desferal (desferrioxamine mesylate), a specific iron chelating agent used in therapy for treating iron toxicity [11]. Further, it is shown that both $OH^{\cdot}$ and H_2O_2 are produced in the system and are the probable cause of the observed deterioration in cardiac function. It is also shown that the superoxide radical ($O_2^{\cdot -}$) is produced but appears to be acting through other oxygen species mentioned above.

Adult male Sprague–Dawley rats weighing 225 ± 25 g were used in the present study. The animals were killed by decapitation, and their hearts were rapidly excised and placed in an ice-cold oxygenated buffer solution. The hearts were mounted on a steel cannula and perfused through the coronary arteries at a constant flow rate (8 ml/min) and temperature (37°) according to the Langendorff method as described previously [12]. The perfusion medium was a modified Krebs Henseleit (KH) solution containing (mM): NaCl, 120; $NaHCO_3$, 25.4; KCl, 4.8; KH_2PO_4, 1.2; $MgSO_4$, 0.86; $CaCl_2$, 1.25; and glucose 11.0. This solution, pH 7.4, was bubbled with a mixture of 95% O_2 and 5% CO_2. The solutions were made in double-distilled water, and no special precaution was taken to remove trace metals from chemical reagents. The hearts freed of all atrial tissue were electrically paced with supramaximal stimuli of 1.5-msec duration at the rate of 350 beats/min. A resting tension of 1 g was applied to the hearts. Myocardial contractile force and rate of change of developed tension were recorded on a Beckman recorder via force displacement transducer (FT 0.03) and a dF/dt coupler.

All the force data at time 0 were normalized to 100%, and subsequent values were expressed as a percent change. A one-way analysis of variance was done to test for any differences among the groups at various time intervals. The data showing any difference were compared with the xanthine–xanthine oxidase group using Tukey's (Q) test [13] for repeated measures.

After a 15-min stabilization period, the hearts were exposed to oxygen radicals generated by the addition of xanthine (2 mM; Sigma)–xanthine oxidase (10 units/l; Calbiochem) according to the reaction:

$$\text{Xanthine} + O_2 + H_2O \xrightarrow{\text{Xanthine oxidase}} O_2^{\cdot -} + H_2O_2 + \text{Uric acid} \quad \text{(IV)}$$

Perfusion of the hearts with the solution containing X–XO with no added iron resulted in contractile failure and a rise in resting tension (Fig. 1). The decline in force was more than 50% in 4 min, and no contractile force was apparent at 20 min (Table 1). Both positive dF/dt and negative dF/dt showed similar changes (Table 2). Inclusion of xanthine or xanthine oxidase alone in the perfusion medium had no effect on these parameters. Although we did not add iron to our system, it can be argued that iron may have been present *in vitro* as a contaminant as well as *in vivo* in trace amount in the heart cells. Since X–XO induced contractile failure in perfused rabbit interventricular septal preparation has been suggested to be predominantly due to $OH^{\cdot}$ produced in the presence of iron [10], we repeated our studies in the presence of desferal (300 μM and 3 mM; Ciba-Geigy), a specific iron chelating agent. These experiments did not reveal any protective effect on X–XO induced contractile failure (Tables 1 and 2). The hearts perfused with desferal (300 μM, 3 mM) alone did not show any change in force over a period of 20 min (N = 4), indicating

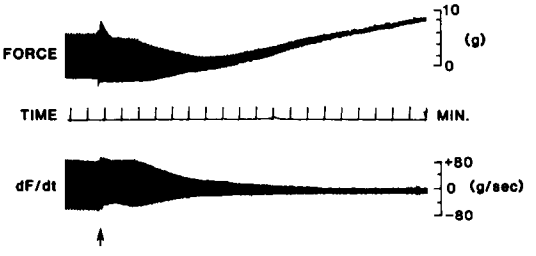

Fig. 1. Typical trace showing effects of xanthine–xanthine oxidase (X–XO) on the profiles of developed force and dF/dt in an isolated perfused rat heart. Arrow indicates introduction of X–XO.

Table 1. Effects of different agents on xanthine–xanthine oxidase (X–XO) induced changes in contractile force of isolated perfused rat heart at various time intervals

Agent	Concentration	Contractile force (%)				
		Perfusion time (min)				
		4	8	12	16	20
X–XO	2 mM; 10 units/l	49 ± 7	24 ± 4	12 ± 3	4 ± 4	0
X–XO + Desferal	300 μM	48 ± 3	16 ± 3	7 ± 1	3 ± 2	0
	3 mM	50 ± 2	25 ± 2	13 ± 1	6 ± 2	0
X–XO + Superoxidase dismutase	3×10^4 units/l	43 ± 4	23 ± 3	14 ± 1	6 ± 1	0
	1.2×10^5 units/l	81 ± 4*	71 ± 6*	65 ± 9*	58 ± 10*	56 ± 10*
X–XO + Mannitol	10 mM	85 ± 2*	67 ± 2*	58 ± 3*	52 ± 2*	44 ± 1*
	20 mM	91 ± 2*	80 ± 7*	72 ± 19*	68 ± 10*	66 ± 12*
X–XO + Catalase	2×10^4 units/l	84 ± 3*	74 ± 2*	58 ± 2*	44 ± 2*	35 ± 2*
	4×10^4 units/l	83 ± 2*	76 ± 2*	74 ± 1*	71 ± 1*	65 ± 2*

Values are mean ± SEM of five experiments and are expressed as percent of zero time control data. For a representative control contractile force value, see Fig. 1.

* Significantly different ($P < 0.01$; ANOVA one-way; Q test) from hearts perfused with Krebs Henseleit solution containing X–XO. Contractile force in the absence of X–XO was stable for more than 1 hour.

Table 2. Effects of different agents on xanthine–xanthine oxidase (X–XO) induced changes in positive (+) and negative (−) dF/dt in isolated perfused rat hearts

		Rate of change in contractile force (dF/dt)									
		Perfusion time (min)									
		4		8		12		16		20	
Agent	Concentration	+	−	+	−	+	−	+	−	+	−
X–XO	2 mM; 10 units/l	50 ± 6	51 ± 5	27 ± 4	23 ± 4	12 ± 3	10 ± 2	5 ± 1	0.5 ± 0.5	0	0
X–XO + Desferal	300 μM 3 mM	51 ± 9 42 ± 11	39 ± 1 47 ± 7	28 ± 2 29 ± 4	20 ± 1 24 ± 3	12 ± 2 12 ± 1	11 ± 1 12 ± 4	6 ± 2 5 ± 1	0 2 ± 6	0 0	0 0
X–XO + Superoxide dismutase	3×10^4 units/l 1.2×10^5 units/l	47 ± 2 84 ± 2*	28 ± 2 81 ± 3*	22 ± 1 77 ± 5*	9 ± 2 72 ± 6*	14 ± 1 71 ± 7*	6 ± 2 66 ± 8*	6 ± 2 68 ± 8*	2 ± 2 61 ± 9*	0 58 ± 9*	0 51 ± 9*
X–XO + Mannitol	10 mM 20 mM	81 ± 1* 93 ± 3*	85 ± 2* 91 ± 2*	68 ± 3* 91 ± 5*	74 ± 1* 80 ± 5*	64 ± 1* 85 ± 6*	68 ± 3* 74 ± 6*	55 ± 1* 76 ± 13*	63 ± 3* 71 ± 9*	52 ± 3* 69 ± 12*	53 ± 2* 69 ± 12*
X–XO +	2×10^4 units/l 4×10^4 units/l	90 ± 2* 87 ± 3*	88 ± 2* 77 ± 7*	78 ± 4* 84 ± 3*	70 ± 6* 63 ± 3*	57 ± 2* 77 ± 2*	52 ± 4* 62 ± 4*	43 ± 3* 69 ± 2*	45 ± 2* 54 ± 6*	32 ± 1* 66 ± 2*	31 ± 2* 52 ± 6*

Values are mean ± SEM of five experiments, expressed as percent of zero time control data. For a representative control dF/dt value, see Fig. 1.

* Significantly different ($P < 0.01$; ANOVA one-way; Q test) from hearts perfused with Krebs Henseleit solution containing X–XO. Both + and − dF/dt in the absence of X–XO were stable for more than 1 hour.

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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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.