was 15% and 25%; both formulations showed a repellent activity on the lepidoptera larvae treated by ingestion, whereas only **5b** caused mortality, in 10% of individuals in the contact tests. Compounds **5c** and **5d** were active only on *T. mori* (30% and 20%). The most active were **5f** and **5g**; both were active on 20% of the individuals treated; hatching inhibition of *B. mori* eggs was 13% and 15% respectively; **5g** caused mortality in 20% of the contact-treated larvae of this species. It was very interesting to observe the activity on pupae where 30% and 40% respectively of treated emerged adults presented malformations (figure). Furthermore, **5g** inhibited emergence in 20% of pupae.

Group 6 compounds: the formulation **6a** had caused slight juvenoid effects on *T. molitor* in 20% of the population treated; subsequently tested on *B. mori* this formulation inhibited hatching in 10% of the eggs and had a repellent effect on the ingestion-treated larvae. Compounds **6f** and **6g** were both active on *T. molitor* in 10% of the cases and on *B. mori* eggs in 13% and 10% of the cases, respectively; furthermore the former induced malformations in adult lepidoptera emerged from 10% of treated pupae whilst the latter caused mortality in 10% of the contact treated larvae.

Group 7 compounds: slight malformations in *T. molitor* were induced by **7a** and **7g** (10%) and **7f** (15%). Tests on *T. molitor* for inhibition of emergence showed that **7g** (14%) and particularly subjected to contact tests malformations in 20% of the adults and emergence inhibition in 20% of the eggs.

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Effects of scavengers of superoxide radicals, hydrogen peroxide, singlet oxygen and hydroxyl radicals on malondialdehyde generation from arachidonic acid by bovine seminal vesicle microsomes

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Summary. Superoxide dismutase, catalase and sodium formate did not inhibit the formation of malondialdehyde (MDA) from arachidonic acid, suggesting that $O_2^{\cdot}$, H_2O_2 and $OH^{\cdot}$ are not involved in the enzymatical oxidation of arachidonic acid. Sodium azide was found to be an inhibitor of MDA production.

Since the observation by Babior et al.[1] that phagocytosing polymorphonuclear leukocytes, effector cells of the acute inflammatory response, release large amounts of superoxide anions ($O_2^{\cdot}$) into the medium in which the activated cells are suspended, evidence has been found for the possible involvement of oxygen species as mediators of inflammation. In 1971 Vane[2] proposed that the anti-inflammatory action of aspirin-like drugs is based on their ability to inhibit prostaglandin (PG) biosynthesis. After that, many authors reported findings supporting the idea that mediation of inflammation is correlated with lipid peroxidation[3,4], in general, and with oxygenation of arachidonic acid[2,5-9] in particular. In addition, anti-oxidants have been demonstrated to inhibit lipoxidase[10] and PG synthetase[10], and to potentiate the anti-inflammatory action of indometacin[11]. The following question has become a question of considerable interest; what oxygen species is (are) implicated in peroxidation of polyunsaturated lipids and in oxygenation of arachidonic acid[5,6,12,13]?

Linolenate was found to be peroxidized by xanthine oxidase acting aerobically upon acetaldehyde[12]. Superoxide dismutase (SOD) as well as catalase inhibited this lipid peroxidation, indicating that both $O_2^{\cdot}$ and hydrogen peroxide (H_2O_2) were essential intermediates. Scavengers of singlet oxygen ($^1O_2$) also inhibited the peroxidation of linolenate, whereas agents known to scavenge hydroxyl radicals ($OH^{\cdot}$) did not. Therefore, $^1O_2$ was proposed to be responsible for linolenate peroxidation.

The oxidation of arachidonic acid has been considered to require H_2O_2 by some authors[5] and $O_2^{\cdot}$ by others[6].

This paper deals with the effect of SOD, catalase, sodium formate and sodium azide on the in vitro production of malondialdehyde by PG synthetase from bovine seminal vesicles, in order to discover which oxygen species is involved in the peroxidation of arachidonic acid.

Materials and methods. Chemicals. Superoxide dismutase (2800 units/mg protein, from bovine blood) and catalase (3600 units/mg protein, from beef liver) were purchased from Sigma Chemical Co., St. Louis, USA. Methylene blue was obtained from E. Merck, Darmstadt, G.F.R. All other chemicals and organic solvents were of analytical grade and purchased from J.T. Baker Chemicals B.V., The Netherlands.

Preparation of prostaglandin synthetase. PG synthetase was prepared from bovine seminal vesicles essentially according to the method of Takeguchi et al.[14]. The precipitated microsomes were suspended (not lyophilized) in 50 mM Tris-HCl buffer (pH = 8.3) and stored at −20 °C in small containers. The yield of microsomal protein from 1414 g of vesicles was 20.8 g, as determined by the method of Lowry et al.[15].

Inhibition of malondialdehyde formation. The convenience

and sensitivity of the thiobarbituric acid test have led to its widespread use in assessing lipid peroxidation. However, its specificity is questionable. Thiobarbituric acid-reactive breakdown products may also arise from the thermal or acid decomposition of endoperoxides during the test[16,17]. In control experiments we have shown that this did not increase significantly the color yield resulting from malondialdehyde (MDA) formed enzymatically.

The determination of MDA formation was based on methods described by Takeguchi et al.[14] and by Flower et al.[18]. PG synthetase, 3.84 mg of microsomal protein, 0.75 mM arachidonic acid, 1 mM glutathione, 1 mM epinephrine and the test compound were incubated at 37 °C for 10 min in 50 mM Tris-HCl buffer (pH = 8.3). The final volume was 3 ml. The reaction was terminated by addition of 0.5 ml of 100% trichloroacetic acid (w/v) in 1 N HCl. 0.5 ml of a 1% solution of sodium thiobarbiturate was added, and the color from its reaction with MDA was developed by heating at 100 °C for 20 min. After centrifugation, the absorbance was measured at 532 nm; a zero-time control was the spectrophotometric blank. The MDA concentrations were determined from a standard curve employing malondialdehyde bis(dimethylacetal).

Results and discussion. A number of compounds known to react with highly reactive species of oxygen and oxygen-related agents, were tested for their ability to inhibit MDA formation. The effects of the scavengers are shown in the table. SOD and catalase did not inhibit the formation of MDA, suggesting that $O_2^{\cdot-}$ and H_2O_2 are not involved in the oxidation of arachidonic acid.

In aerated solutions formate converts $OH\cdot$ to $O_2^{\cdot-}$[19–21]:

$$OH\cdot + HCOO^- \rightarrow H_2O + CO_2^{\cdot-}$$

$$CO_2^{\cdot-} + O_2 \rightarrow CO_2 + O_2^{\cdot-}$$

Formate up to 50 mM had no effect on MDA generation. This finding, together with the lack of inhibition by SOD, indicates that $OH\cdot$ was not involved in the oxidation of arachidonic acid either.

In contrast to our in vitro findings, Oyanagui[6] suggested that $O_2^{\cdot-}$ are involved in the in vivo oxidation of arachidonic acid. This author based his conclusion on the fact that after i.v. administration of SOD to rats the PG phase swelling was completely suppressed. In our opinion, this effect cannot only be explained by inhibition of the PG biosynthesis. It was demonstrated that the carrageenan-induced foot oedema in essential fatty acid deficient rats could not be reduced by indometacin[22]. On the contrary, aspirin did exhibit inhibition[22]. The bradykinin component of inflammation was shown to last for at least 5 h[23,24]. Hence, complete inhibition of PG biosynthesis does not play a central role in the suppression of the oedema.

Vargaftig et al.[5] have shown that catalase inhibited platelet aggregation and the generation of rabbit-aorta contracting and PG-like activities, when platelets were challenged with arachidonic acid. Comparing these findings with the results of our experiments with catalase it might be concluded that catalase did not inhibit cyclo-oxygenase.

Sodium azide is considered to be an effective scavenger of $^1O_2$[25–27]. This compound was found to be an inhibitor of the MDA formation (see table). However, addition of methylene blue, a $^1O_2$ generator[28–30], at a concentration of 10^{-7} M to the incubation medium did not enhance the MDA production. Combined with the fact that sodium azide quenches $^1O_2$ very efficiently (at 5×10^{-4} M the rate constant for the quenching is 2.2×10^8 M^{-1} sec^{-1} [26]), it should be concluded that $^1O_2$ is not involved in the in vitro oxidation either, and azide inhibits one or more of the enzymes catalyzing the oxidation of arachidonic acid.

Effect of SOD, catalase, sodium formate and sodium azide on the formation of MDA by bovine seminal vesicle microsomes

Compound	Concentration	MDA formation expressed as % of control
SOD	5 µg/ml	98.6
	10 µg/ml	98.4
	20 µg/ml	103.3
Catalase	4.16 µg/ml	100.2
	12.48 µg/ml	100.5
	25 µg/ml	98.7
Sodium formate	10 mM	100.3
	15 mM	98
	50 mM	103.8
Azide	5 mM	95*
	10 mM	90.5*
	20 mM	72.8*
	40 mM	49.3*

* $p < 0.05$ (Student's t-test).

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