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PARTICIPATION OF SUPEROXIDE ANIONS AT THE PROSTAGLANDIN PHASE OF CARRAGEENAN FOOT-OEDEMA

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Abstract Bovine superoxide dismutase (SOD: 0.5–2.0 mg/kg) administered intravenously to rats, completely suppressed the prostaglandin phase swelling (2–4 hr) of carrageenan foot-oedema, but had no effect on the histamine and serotonin phase ($\frac{1}{2}$–$1\frac{1}{2}$ hrs.). Heat-inactivated SOD, bovine serum albumin and catalase and high doses of the hydroxyl radical scavengers, sodium benzoate and D-mannitol or the oxygen scavenger, 1,3-diphenylisobenzofuran had no effect on the swelling. Mepylamine plus methysergide did not influence the inhibitory action of SOD. Carrageenan foot-oedema of agranulocyte rats, induced by methotrexate injections, was more susceptible than that of normal rats to SOD inhibition. Even at 1 hr, about 70 per cent inhibition of swelling was observed suggesting the importance of macrophages in this inflammation model. Indomethacin and oxyphenbutazone were also examined for comparison with the effect of SOD. The role of superoxide anions in inflammation is discussed in connection with macrophage emigration, releases of lysosomal enzymes and prostaglandin biosynthesis.

It has been reported [1, 2, 3] that granulocytes produce superoxide anions ($\cdot O_2^-$) to destroy invasive bacteria. Gemsa *et al.* [4] reported endotoxin-stimulated heme oxygenase activity in rat macrophages. The production of superoxide anions in macrophages collected from intraperitoneal exudates of paraffin oil-injected guinea pigs has also been demonstrated [5]. McCord [6] protected bovine synovial fluid degradation by superoxide dismutase (SOD: E.C. 1.15.1.1.) or by catalase and Repine *et al.* [7] observed an augmentation of oxygen consumption, hexose monophosphate shunt activity and reduction of nitroblue tetrazolium in human neutrophils by addition of phorbol myristate which is an effective component of croten oil, a typical inflammation-inducing agent. These biochemical changes suggest the enhanced production of superoxide anions.

On the other hand, the role of prostaglandins (PGs) in inflammation is well discussed and the inhibition of prostaglandin synthetase has been proposed as a good *in vitro* screening method for non-steroidal anti-inflammatory drug. PGs production from incubated polymorphonuclear (PMN) leucocytes is reported by Glatt *et al.* [8] and the level of prostaglandin E in carrageenan (= carrageenin) granuloma is reported to parallel the development of inflammation [9]. Willis *et al.* [10] detected PGs in carrageenan foot-oedema.

The oxidation of arachidonic acid to PGs requires superoxide anions [11]. Panganamala *et al.* [12, 13] reported that the active radical in PGs syntheses is not superoxide but a hydroxyl-like radical derived from superoxide anions. Toxic free radicals produced from superoxide anion, are hydroxyl radical ($\cdot OH$), singlet oxygen ($^1O_2$) and malondialdehyde [3, 14, 15]. The H_2O_2-myeloperoxidase–halide system is also involved in the production of these radicals.

Our investigations have focused therefore on the role of superoxide anions in carrageenan-induced foot-oedema. Direct administration of SOD into the inflamed foot failed to have any anti-inflammatory effect, because SOD, like many anti-inflammatory agents, also possesses irritant properties. Carrageenan is known by electromicroscopic observation to be engulfed into macrophages [16]. Di Rosa *et al.* [17, 18] insisted on the importance of macrophages in carrageenan foot-oedema, but Blackham *et al.* [19] claimed that leucocyte emigration is essential to carrageenan oedema. Vinegar *et al.* [20] distinguished two phases in the swelling of carrageenan foot-oedema. The first phase (1/2–1 1/2 hr) is maintained by the release of histamine and serotonin and the second phase (2–6 hr) by prostaglandins. The Kinins phase (1 1/2–2 1/2) is minor and overlaps the two other phases.

This report concerns the effects of SOD, radical scavengers and anti-inflammatory drugs on rat carrageenan foot-oedema.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats, SLC-strain (190–220 g), were obtained from Sizuoka Agr. Coop. Assoc. for Lab. Animal.

Assay. Carrageenan (0.1 ml in saline), 1.5% (except Fig. 1), was injected into the plantar surface of the hind paw. The control group received saline only. The thickness of the foot was measured in mm with a capiller compass always by the same person and represented the degree of swelling. The foot was cut off for weighing 4 hr after carrageenan injection. Drugs (0.5 ml) were injected intravenouly (i.v.) in general. The group given one injection always received the drug 30 min before carrageenan, the group given two injections received the drug 30 min before and 2 hr after carrageenan and the three-dose group was also injected at 3 hr.

Chemicals. Bovine superoxide dismutase (lyophilized powder) was purchased from Sigma Chem. Co.. Catalase I (50,000 U/mg, beef liver, crystal suspension in thymol saturated water) was the product of Boehringer Co. Sigma bovine blood catalase (3,000 U/mg, lyophilized powder) was used for comparison. SOD was inactivated by boiling the enzyme solution (800 μg/ml) at pH 7.0 and 100° for 20 min. Mepylamine (Pylilamine) hydrochloride (K & K Lab.), methysergide bimaleate (Sandoz Co.) and methotrexate (Lederle Co.) were used as saline solutions. 1,3-Diphenylisobenzofuran (=2,5-diphenyl-3,4-benzofuran, Aldrich Chem. Co.) were injected as a homogenous suspension in 3% *N,N'*-dimethylformamide (DMF) at pH 7.0. All other chemicals were analytical grade preparations obtained from the usual commercial sources.

Calculations. The percentage inhibition of swelling or of foot weight, was calculated by the following formula:

$$\text{Inhibition (\%)} = \left[1 - \frac{\left(\begin{matrix}\text{Value of drug}\\ \text{carrageenan rat}\end{matrix}\right) - \left(\begin{matrix}\text{Value of drug}\\ \text{saline rat}\end{matrix}\right)}{\left(\begin{matrix}\text{Value of control}\\ \text{carrageenan rat}\end{matrix}\right) - \left(\begin{matrix}\text{Value of control}\\ \text{saline rat}\end{matrix}\right)}\right] \times 100$$

When DMF was used as solvent, control values were obtained with a DMF-injected group.

RESULTS

Carrageenan concentration. To test the irritant capacity of the carrageenan, four different concentrations were examined (Fig. 1). A concentration of 1.5% was chosen for the experiment, because high concentrations possibly demand a higher drug dose than is really required and at concentrations of 1.0 or 0.75% the swelling began to decrease at 4 hr. Control values with 1.5% carrageenan solution were estimated in each experiment.

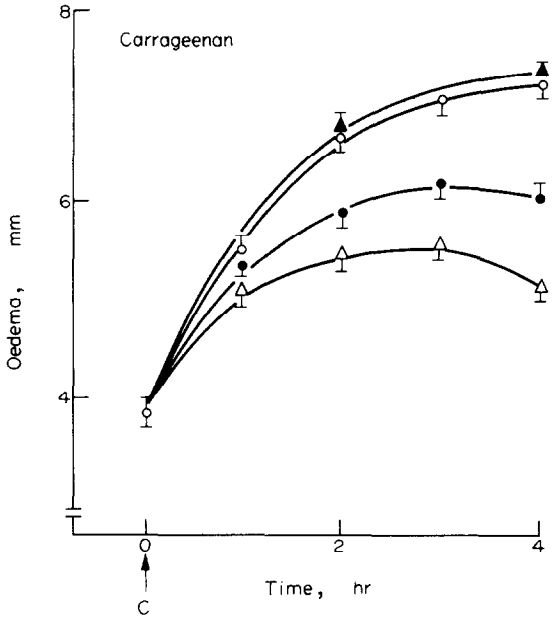

Fig. 1. Foot swelling induced by different concentrations of carrageenan (0.1 ml) at arrow C. 3.0% (▲); 1.5% (○); 1.0% (●) and 0.75% (△). Vertical lines represent the standard errors (S.E.M.) of the mean of 6–10 experiments.

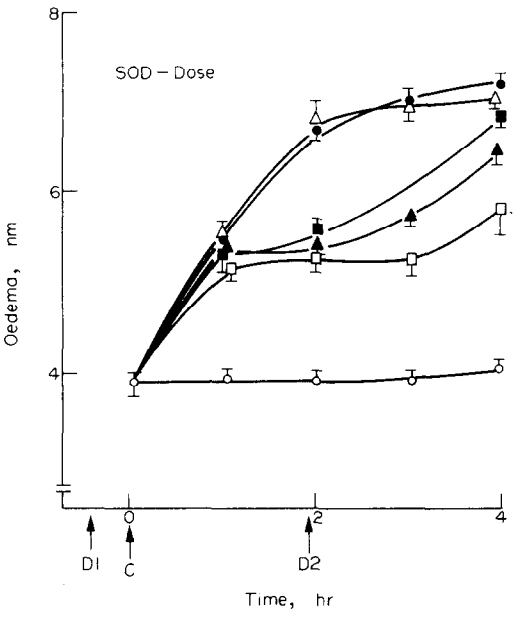

Fig. 2. Effect of two injections of superoxide dismutase (SOD) at D1 and D2 on carrageenan (1.5% solution at C) induced foot-swelling. Saline injected control (●); SOD 0.5 mg/kg (■); 1 mg/kg (▲); 2 mg/kg (□); heat-inactivated SOD 1 mg/kg (△) and saline without carrageenan (○). Vertical lines represent S.E.M. of the mean of at least 4 experiments (exact number is in Tables 1–3).

Inhibition by superoxide dismutase. A linear dose inhibition relationship at 4 hr was obtained between 0.5 and 2.0 mg/kg SOD with two administrations (Fig. 2). Swelling of the first phase (1/2–1 1/2 hr) which is sustained by histamine and serotonin was not influenced by SOD and this enzyme was found to inhibit only the prostaglandin phase (2–4 hr). Heat-inactivated SOD, like bovine serum albumin, did not inhibit significantly the foot swelling (Fig. 2, Table 1). The inhibitory effect of 2 mg/kg SOD was reduced when the second injection was omitted and three injections suppressed completely the swelling of the prostaglandin phase (Fig. 3). SOD seemed to be inactivated in the blood stream or absorbed rapidly by tissues so that repeated administration was necessary for maximum inhibition of swelling. Intravenous administration of SOD had no effect on a normal foot.

Inhibition by manganese ion. Manganese chloride is known as a superoxide radical scavenger [21]. The production of superoxide radicals by guinea pig macrophages was reduced 50 per cent by 4×10^{-6} M manganese ions [5]. The effect of 20 mg/kg manganase chloride was similar to that of 1 mg/kg SOD in reducing the swelling of the prostaglandin phase (Fig. 4).

Effects of catalase and radical scavengers. Hydrogen peroxide produced by superoxide anions could influence foot swelling, but high concentration of liver catalase had no effect (Fig. 5). Blood catalase caused

Table 1. Suppression of carrageenan foot-oedema by superoxide dismutase at 4 hours

Drug	mg/kg i.v.	Injection time*	No. of exp.	Swelling		Wt increased	
				Mean ± S.E.M. (mm)	Inhibition (%)	Mean ± S.E.M. (g)	Inhibition (%)
Control		2	4	3.7 ± 0.1	(0)	0.80 ± 0.03	(0)
SOD	0.5	2	4	3.4 ± 0.2	8	0.63 ± 0.02†	21
SOD	1.0	2	4	2.6 ± 0.3†	29	0.40 ± 0.07†	50
SOD	2.0	2	4	2.1 ± 0.2‡	43	0.37 ± 0.02‡	54
Inactivated SOD	2.0	2	4	3.5 ± 0.1†	5	0.67 ± 0.01†	16
Control		2	10	3.2 ± 0.1	(0)	0.93 ± 0.05	(0)
SOD	1.0	2	4	2.5 ± 0.2‡	22	0.47 ± 0.07‡	49
SOD	2.0	1§	4	2.3 ± 0.1‡	28	0.27 ± 0.05‡	71
SOD	2.0	2	4	1.9 ± 0.3‡	41	0.26 ± 0.02‡	77
SOD	2.0	3§	4	1.4 ± 0.1‡	56	0.18 ± 0.01‡	81
Inactivated SOD	1.0	2	6	3.0 ± 0.1	6	0.83 ± 0.05	11
Control		3	10	3.2 ± 0.1	(0)	0.70 ± 0.12	(0)
SOD	1.0	3	4	1.3 ± 0.1‡	59	0.10 ± 0.04‡	86
(Mp + Ms) only*		3	6	3.0 ± 0.1	6	0.67 ± 0.07	4
(Mp + Ms) + SOD	1.0	3	4	1.9 ± 0.1‡	41	0.34 ± 0.07‡	51
MTX only*		3	8	2.9 ± 0.1	9	0.74 ± 0.06	−6
MTX + SOD	1.0	3	6	1.3 ± 0.3‡	59	0.20 ± 0.04‡	71

* For the time of injection and other conditions, see Materials and Methods. Mp (mepyramine·HCl, 10 mg/kg) and Ms (methysergide·bimaleate, 4 mg/kg) were mixed with SOD solution in every injection. MTX (methotrexate, 2.5 mg/kg/day, i.p.) was given for 3 days. The last injection was 1 hr before carrageenan injection.

† Significantly different from the control at $0.001 < P < 0.05$ by Students *t*-test.

‡ Significantly different from controls ($P < 0.001$).

§ One or three injections of saline solution had the same effect on swelling as the two injection group.

some reduction of swelling after 2 hr. This difference may be due to the different sources of the catalase preparations as Halliwell *et al.* [22] demonstrated in *in vitro* assay. Blood catalase seems to contain SOD. Hydroxyl radical (·OH) generated from superoxide radical and hydroxygen peroxide, may be a directly acting swelling factor. High doses of sodium benzoate and D-mannitol, both of which are typical hydroxyl

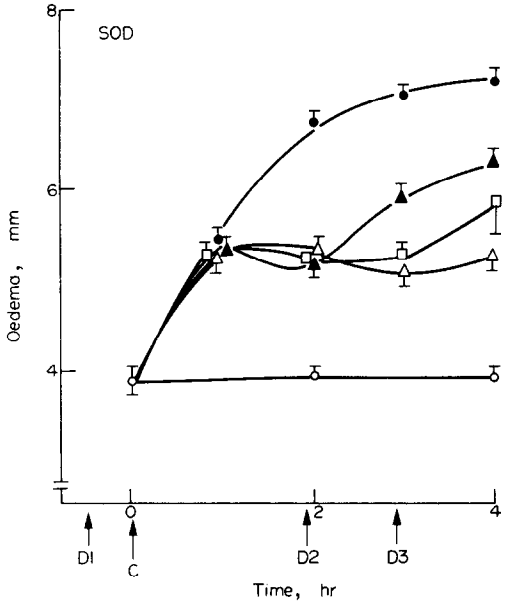

Fig. 3. Effect of injection times of SOD (2 mg/kg). Two saline injections, control (●); one injection of SOD at D1 (▲); two injections of SOD at D1 and D2 (□); three injections of SOD at D1, D2 and D3 (△) and two injections of SOD at D1 and D2 without carrageenan (○). Other indications as in Fig. 1 and Fig. 2.

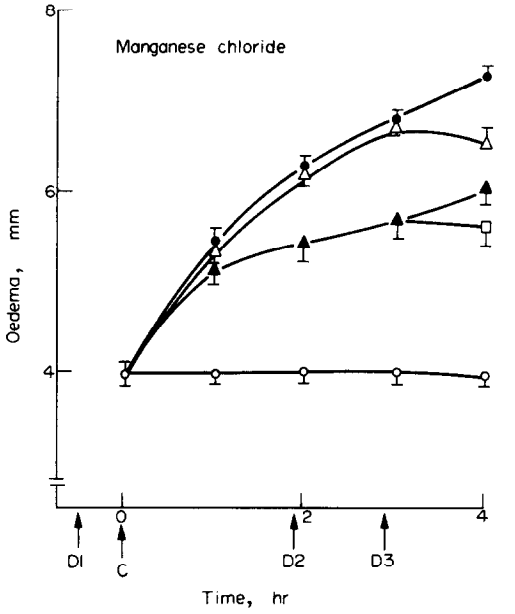

Fig. 4. Effect of manganese chloride. Two saline injections, control (●); two injections of $MnCl_2$ 10 mg/kg (△); two injections of 20 mg/kg (▲); three injections of 20 mg/kg (□); and two injections of 20 mg/kg without carrageenan (○). Other indications as in Fig. 1 and Fig. 2.

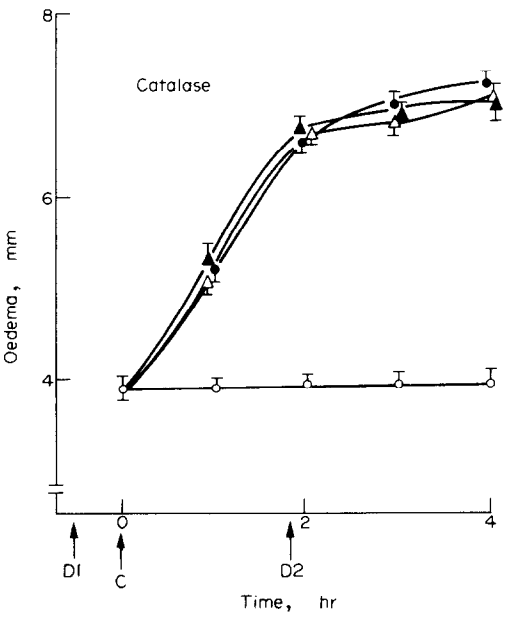

Fig. 5. Effect of two injections of bovine liver catalase. Saline control (●); catalase 240,000 U/kg (△), 960,000 U/kg (▲) and 960,000 U/kg without carrageenan (○). Other indications as in Fig. 1 and Fig. 2.

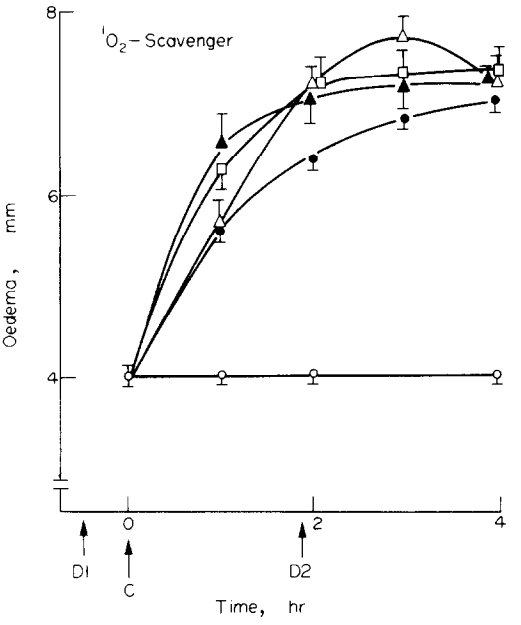

Fig. 7. Effect of two injections of the singlet oxygen scavenger, 1,3-diphenylisobenzofuran dissolved in 3% DMF. 5 mg/kg (△); 10 mg/kg (□); 20 mg/kg (▲) and 10 mg/kg without carrageenan (○); 3% DMF, control (●). Other indications as in Fig. 1 and Fig. 2.

radical scavengers, had no effect (Fig. 6). Singlet oxygen ($^1O_2$) is not a direct acting radical, because a singlet oxygen scavenger, 1,3-diphenylisobenzofuran [23] did not inhibit the oedema (Fig. 7). On the contrary, this agent enhanced the swelling of carrageenan foot-oedema. The details of this mechanism remain a problem.

Effects of antagonists of histamine and serotonin. The first swelling phase (1/2–1 1/2 hr) was not influenced by the antagonists of histamine, mepyramine (10 mg/kg) and of serotonin, methysergide (4 mg/kg), but when they were mixed about fifty per cent inhibition was observed at 1 hr (Fig. 8). Inhibition of swelling by this mixed solution was slight after 3 hr despite

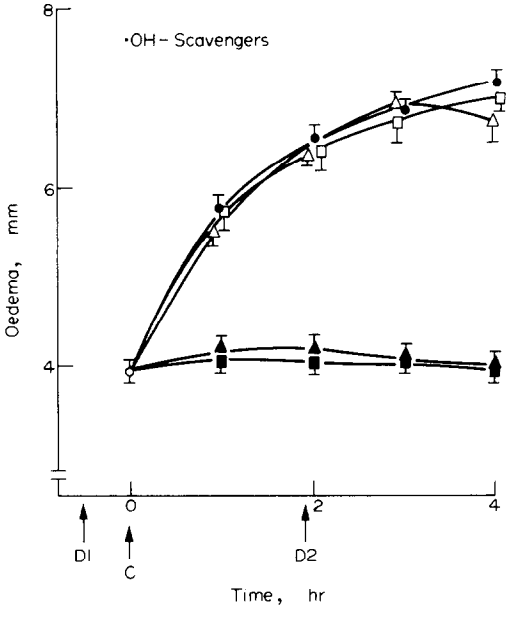

Fig. 6. Effects of two injections of hydroxyl radical scavengers. Saline control (●); sodium benzoate 160 mg/kg with (△) and without (▲) carrageenan; D-mannitol 400 mg/kg with (□) and without (■) carrageenan. Other indications as in Fig. 1 and Fig. 2.

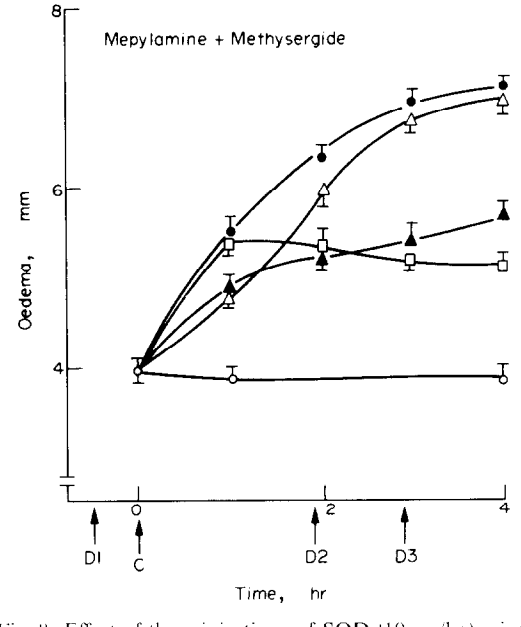

Fig. 8. Effect of three injections of SOD (10 mg/kg) mixed with mepylamine·HCl (10 mg/kg) plus methysergide bimaleate (4 mg/kg). Saline control (●); mepylamine plus methysergide (△); SOD (□); SOD and mepylamine plus methysergide with (▲) and without (○) carrageenan. Other indications as in Fig. 1 and Fig. 2.

repeated administration at 2 hr and 3 hr (Fig. 8, Table 2). Three injections of a mepyramine (10 mg/kg), methysergide (4 mg/kg) and SOD (1 mg/kg) mixture, reduced the swelling by about 50 per cent at 1 hr, but the suppression of the prostaglandin phase by SOD was gradually resumed. This phenomenon may be explained by recompense swelling owing to strong inhibitions of the first phase. The mepyramine plus methysergide group also showed accelerated swelling speed after 1 hr. The same tendency was observed when 10 mg/kg indomethacin was administered before the carrageenan injection (Fig. 11). However, it is evident that SOD inhibits the prostaglandin phase under the influence of mepyramine plus methysergide.

The concentrations of mepyramine (10 mg/kg) plus methysergide (4 mg/kg) used induced temporary convulsions in the rat so that an increase in the concentrations in order to inhibit completely the first phase was not possible. Treatment with compound 48/80 to deplete histamine and with cellulose sulphate to deplete kinins, may be a way to abolish completely the first phase. Nevertheless, even with such treatment, the recompense swelling acceleration after 1 hr may still appear and may interfere with the analysis of the SOD effect.

Effect of methotrexate treatment. Agranulocyte rats were produced by intraperitoneal injections of methotrexate for three days [17]. More than 95 per cent of the cells found at the carrageenan injected site at

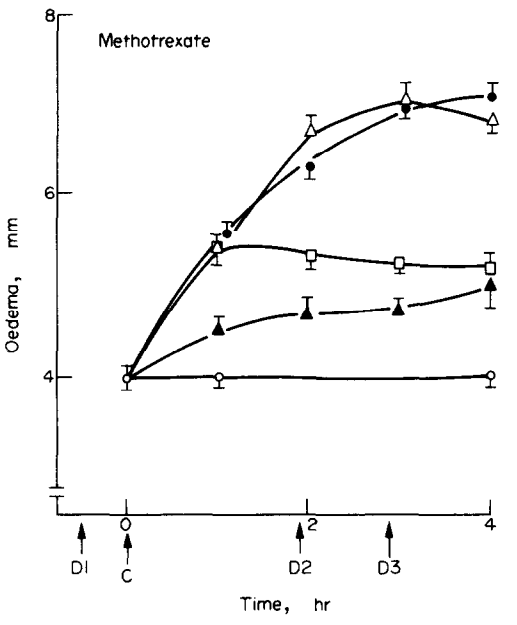

Fig. 9. Effect of three injections of SOD (1 mg/kg) on methotrexate treated rats. Saline injected normal control (●); methotrexate treated control (△); normal rat, SOD (□), methotrexate treated, SOD with (▲) and without (○) carrageenan. Other indications as in Fig. 1 and Fig. 2. See also the legend of Table 1.

Table 2. Suppression of carrageenan foot-oedema by various agents at 4 hours

Drug	mg/kg i.v.	Injection time*	No. of exp.	Swelling Mean ±S.E.M. (mm)	Swelling Inhibition (%)	Weight increased Mean ±S.E.M. (g)	Weight increased Inhibition (%)
Control		2	8	3.3 ± 0.1	(0)	0.98 ± 0.11	(0)
$MnCl_2$	10	2	4	2.5 ± 0.2†	24	0.68 ± 0.03†	31
	20	2	4	2.1 ± 0.2‡	36	0.72 ± 0.05†	27
	20	3	4	1.8 ± 0.2‡	55	0.58 ± 0.12†	41
1,3-diphenyl-isobenzofuran	5	2	4	3.4 ± 0.3	−3	1.02 ± 0.12	−4
	10	2	4	3.6 ± 0.2	−9	1.08 ± 0.04	−10
	20	2	4	3.4 ± 0.1	−3	0.95 ± 0.07	3
Control		2	6	3.2 ± 0.1	(0)	0.69 ± 0.06	(0)
Sodium benzoate	40	2	4	2.9 ± 0.2	9	0.60 ± 0.19	13
	160	2	4	2.7 ± 0.2	16	0.52 ± 0.09	25
D-Mannitol	50	2	4	2.8 ± 0.1	12	0.67 ± 0.03	3
	400	2	4	3.1 ± 0.1	3	0.78 ± 0.09	−13
Bovine Serum Albumin	80	2	4	3.0 ± 0.1	6	0.57 ± 0.07	17
Control		2	6	3.0 ± 0.1	(0)	0.72 ± 0.04	(0)
Boehringer liver catalase	240,000 U/kg	2	4	3.1 ± 0.2	6	0.75 ± 0.06	−4
	960,000 U/kg	2	4	3.2 ± 0.1	3	0.67 ± 0.03	7
Control		3	10	3.2 ± 0.1	(0)	0.70 ± 0.12	(0)
Mp*	10	3	4	3.2 ± 0.1	0	0.86 ± 0.08	−22
Ms*	4	3	4	2.7 ± 0.1†	16	0.62 ± 0.03	11

* See Materials and Methods.
Mp: mephylamine·HCl, Ms: methysergide·bimaleate
†‡ As in Table 1.

Table 3. Suppression of carrageenan foot-oedema by anti-inflammatory drugs at 4 hours

Drug	mg/kg	Injection time*	No. of exp.	Swelling Mean ±S.E.M. (mm)		Swelling Inhibition (%)	Weight increased Mean ±S.E.M. (g)		Weight increased Inhibition (%)
Control		2	8	3.3 ± 0.1	(0)		0.98 ± 0.11	(0)	
3% DMF (i.v.)		2	6	3.2 ± 0.2	3	(0)	0.97 ± 0.14	1	(0)
10% DMF (i.v.)		2	6	3.0 ± 0.1	9		0.79 ± 0.05†	19	
Indomethacin in 3% DMF	10	2	4	2.0 ± 0.1‡		37	0.72 ± 0.14†		26
(i.v.)	20	2	4	1.8 ± 0.1‡		44	0.67 ± 0.10†		31
	10	1	4	2.8 ± 0.2‡		12	0.78 ± 0.11		20
	10	3	4	1.9 ± 0.2‡		41	0.77 ± 0.04†		21
(i.p.)	10	2	4	2.0 ± 0.1‡		37	0.80 ± 0.13		17
	20	2	4	1.8 ± 0.3‡		44	0.72 ± 0.06†		26
Oxyphen-butazone in	10	2	4	2.0 ± 0.2‡		37	0.76 ± 0.07†		22
3% DMF (i.v.)	20	2	4	1.9 ± 0.3‡		41	0.65 ± 0.05†		33

* See Materials and Methods.
†,‡ As in Table 1.

4 hr and 6 hr were macrophages. In the normal rat only about 30 per cent of the cells were macrophages, the rest being PMN leucocytes. The swelling of carrageenan oedema in agranulocyte rats was not very different from that in normal rats (Fig. 9), yet the response to SOD was different. Already at 1 hr, SOD inhibited the swelling by about 70 per cent in agranulocyte rats. As there is little leucocyte in agranulocyte rat, the swelling at 1 hr is probably sustained mainly by the prostaglandin phase instead of histamine or serotonin. Strong inhibition by SOD continued for 4 hr suggesting that macrophages are more sensitive to SOD than leucocytes.

Mason *et al.* [24] found that the arachidonate in PMN leucocytes is only 3% of the total fatty acids, but in macrophages it constitutes about 20 per cent. eration by macrophages was significantly inhibited by these drugs [5]. It is tempting to attach importance to the role of macrophages in carrageenan foot-oedema from the results of Fig. 9 and of *in vitro* macrophage experiments.

Effects of anti-inflammatory drugs. The effects of indomethacin and oxyphenbutazone were compared with those of SOD. Three percent DMF used as solvent, had no effect on the swelling but 10% DMF was slightly inhibitory (Fig. 10, Table 3). Two injections of indomethacin (10 mg/kg and 20 mg/kg) produced about 40 per cent inhibition throughout the observation period (Fig. 10). Three injections of indomethacin (10 mg/kg) resulted in a little stronger inhibition at 4 hr, and almost no inhibition was observed Malondialdehyde which is also a toxic free radical

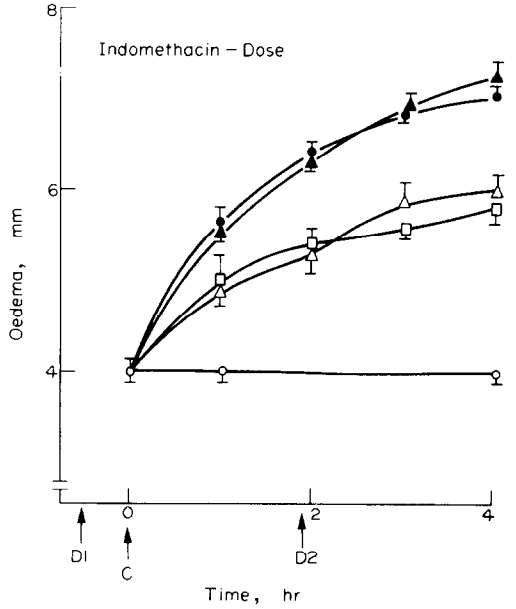

Fig. 10. Effect of two injections of indomethacin dissolved in 3% DMF. Saline control (▲); 3% DMF control (●); indomethacin 10 mg/kg (△), 20 mg/kg (□) and 3% DMF without carrageenan (○). Other indications as in Fig. 1 and Fig. 2.

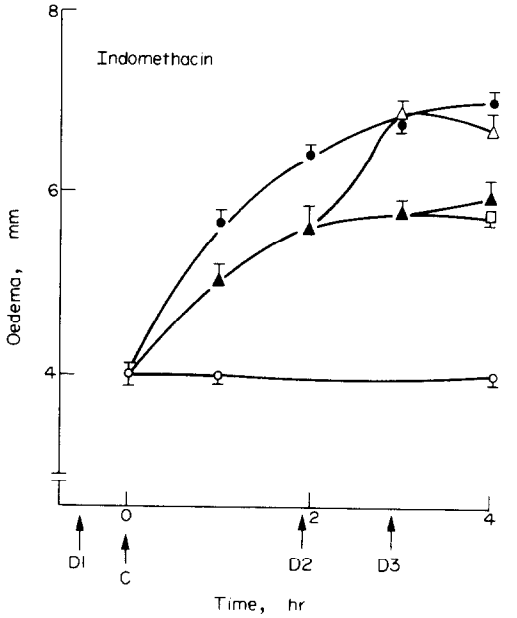

Fig. 11. Effect of indomethacin (10 mg/kg), 3% DMF twice injected control (●); one injection of indomethacin (△); two injections (▲); three injections (□) and two injections without carrageenan (○). Other indications as in Fig. 1 and Fig. 2.

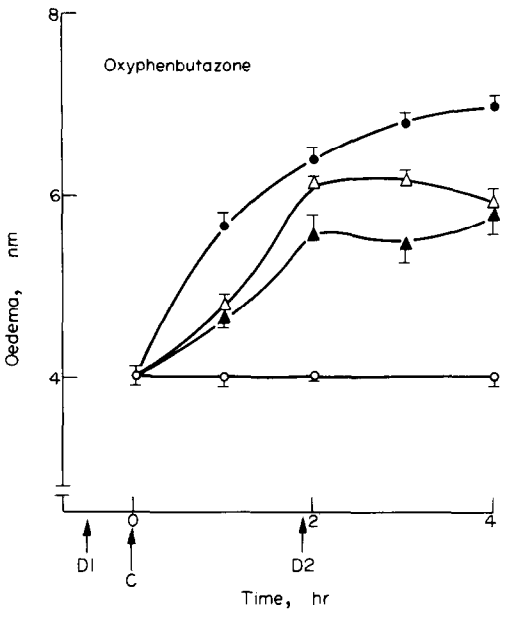

Fig. 12. Effect of two injections of oxyphenbutazone dissolved in 3% DMF. 3% DMF control (●); oxyphenbutazone 10 mg/kg (△), 20 mg/kg with (▲) and without (○) carrageenan. Other indications as in Fig. 1 and Fig. 2.

produced from superoxide anion, was detected only in macrophages. We have not yet examined the effects of anti-inflammatory drugs on the generation of superoxide anions in isolated leucocytes, but the gen-

after 3 hr in the group given one injection (Fig. 11). The inhibition of carrageenan foot-oedema by oxyphenbutazone is shown in Fig. 12.

DISCUSSION

It is difficult to demonstrate directly the increase in superoxide anions in an inflamed region, because this radical is extremely active and reacts with tissues or with foreign substances. Histochemical detection may be possible if a suitable reactant can be found to combine selectively with superoxide anions. The use of SOD, nevertheless, demonstrates the involvement of superoxide anions in carrageenan foot-oedema; only the prostaglandin phase was inhibited suggesting the inhibition of prostaglandin biosynthesis. Other models of experimental inflammation such as ultra-violet erythema, adjuvant arthritis or metal mercury oedema [25], are future projects. Morley *et al.* [26] proposed a hypothesis that macrophages producing PGs regulate the release of lymphokines from lymphocytes. If this kind of mechanism exists, the participation of superoxide anions is also possible in an immunologically induced inflammation. The reactive site of SOD is not clear from our results, but it is probably on macrophages either in the blood stream or at the inflamed site. Superoxide production by isolated macrophages is inhibited by many non-steroidal anti-inflammatory drugs [5].

Davies *et al.* [27] reported the action of carrageenan on the compliment system ten years ago and Giroud *et al.* [28] showed that inflammatory irritants stimulate the complement system resulting in histamine release and fatty acid production serving to form PGs through lecithinase activation. The supply of PGs substrates is naturally important, but they are no more essential rate-limiting factors than superoxide anions in the formation of PGs. Malmsten *et al.* [29] demonstrated that the endoperoxide PGG_2 was more important than prostaglandins E_2 or $F_{2\alpha}$ in the aggregation of human platelets. Arachidonate is transformed via two main pathways: (1) by lipoxygenase to hydroxyeicosatetraenoic acid (HETE) (2) by a cyclo-oxygenase into endoperoxide PGG_2. Prostaglandins E_2 and $F_{2\alpha}$ are produced from PGG_2 and very small amounts of arachidonate resulted to form prostaglandin E_2 and $F_{2\alpha}$. Indomethacin and aspirin inhibit endoperoxide synthesis and not the conversion of PGG_2 to PGE_2 and $PGF_{2\alpha}$.

PGs act as a chemotactic factors as well as increasing vascular permeability [30]. The other lipid oxides can induce chemotaxis of PMN leucocyte [31]. Considering the available information, an inflammatory reaction cycle is proposed, designed partially according to Root *et al.* [36] (Fig. 13). The mechanism of superoxide production in leucocytes or in macrophages is not clear, but the initiation of the production does not always require phagocytosis of bacteria

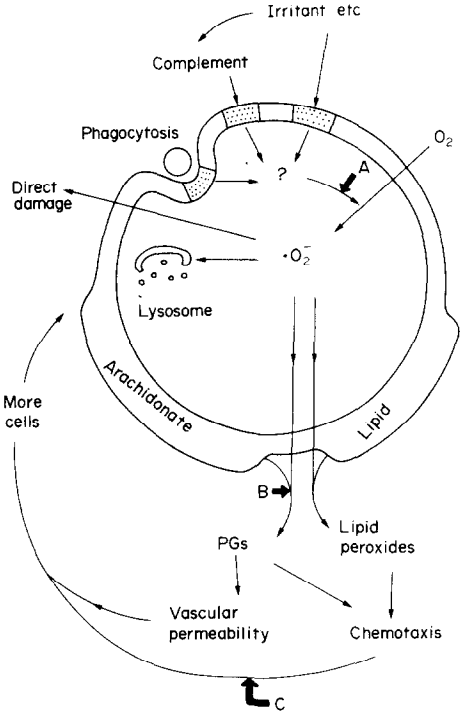

Fig. 13. A concept of the inflammation mechanism. Irritants such as carrageenan, bacteria and perhaps antigen antibody complexes activate unknown system of macrophages or leucocytes to generate superoxide anions. This mechanism is direct and/or through a compliment system and phagocytosis. Generated superoxide anions overwhelm the capacity of endogenous cytoplasmic SOD activity and act on membrane arachidonate or other lipids to produce prostaglandins (PGs) or lipid peroxides. Superoxide anions attack lysosomal membranes and may be released to react with extracellular foreign bodies. PGs and lipid peroxides attract more macrophages or leucocytes to the inflammed site by their chemotactic and vascular permeability increasing characters. A, B and C indicate the sites of inhibition by anti-inflammatory drugs. See also the text.

etc. The contact of leucocytes with cytochalasin E induced superoxide production [21]. Superoxide anions once generated in large amounts, may attack directly the tissues which do not contain sufficient endogenous SOD. Denaturation of proteins and enzymes *in vitro* by superoxide anions was reported by Lavelle *et al.* [32]. Peroxidation of lysosomal membranes by hydroxyl radicals resulting in the rupture of the membranes and release of the lysosomal enzymes was reported by Fong *et al.* [15], but it is difficult to explain the inflammation by the release of lysosomal enzymes alone. In many investigations on the lysosomal membrane stabilizing effects of anti-inflammatory drugs, high doses of agents were used. The release of lysosomal enzymes by superoxide anions did not seem to be the main reason for the development of inflammation. The cycle, macrophages (leucocytes) → superoxide anions → PGs (lipid peroxides) → chemotaxis (vascular permeability increase) → more macrophages (leucocytes) shown in Fig. 13, is the major scheme in the development of inflammation. In a following paper we report that site A is a point where many non-steroidal anti-inflammatory drugs act [5]. Site B is the site of inhibition of PGs biosynthesis. Site C is related mainly to steroid action which inhibits the cell migration. Whilhelmi [33] reported that butazolidin and indomethacin as well as prodonisone, suppress the chemotaxis of cells in formalin peritonitis. Inhibition of chemotaxis *in vivo* by non-steroidal anti-inflammatory drugs must be a reflection of chemotactic PGs deficiency. According to Rinehart [34], steroids inhibit only the emigration of macrophages and not of leucocytes. Their results are in accordance with the concept that macrophages play a more important role than leucocytes in many type of inflammations.

Anti-inflammatory drugs may also compete with PGs for PGs receptor(s) and the inhibition of PGs release from tissue, as supposed by Lewis *et al.* [35] for steroids in adipose tissue. These effects are equivalent to the inhibition of PGs biosynthesis or chemotactic suppression *in vivo*. When the immunological concept is introduced, Fig. 13 must be supplemented with lymphokins effects etc. There is still a possibility however that superoxide generation is a membrane event [37].

REFERENCES

1. J. T. Curnette and B. M. Babior, *J. clin. Invest.* **53**, 1662 (1975).
2. R. S. Weening, R. Wever and D. Roos, *J. Lab. clin. Med.* **85**, 245 (1975).
3. R. C. Allen, S. J. Yevich, R. W. Orth and R. H. Steele, *Biochem. biophys. Res. Commun.* **60**, 909 (1974).
4. D. Gemsa, C. H. Woo, H. H. Fudenberg and R. Schmid, *J. clin. Invest.* **53**, 647 (1975).
5. Y. Oyanagui, *Biochem. Pharmac.* **25**, 000 (1976).
6. J. M. McCord, *Science* **185**, 529 (1974).
7. J. E. Repine, J. G. White, C. C. Clawson and B. M. Holmes, *J. clin. Invest.* **54**, 83 (1974).
8. M. Glatt, B. Peskar and K. Brune, *Experientia* **30**, 1257 (1974).
9. W.-C. Chang, S. Murota and S. Tsurufuji, *Jap. J. Pharmac.* **25**, 219 (1975).
10. A. L. Willis, *J. Pharm. Pharmac.* **21**, 126 (1969).
11. A. Yoshimoto, H. Ito and K. Tomita, *J. Biochem., Tokyo* **68**, 487 (1970).
12. R. V. Panganamala, N. R. Brownlee, H. Sprecher and D. G. Gornwell, *Prostaglandins* **7**, 21 (1974).
13. R. V. Panganamala, H. M. Sharma, H. Sprecher, J. C. Geer and D. G. Gornwell, *Prostaglandins* **8**, 3 (1974).
14. S. J. Klebanoff, *J. biol. Chem.* **249**, 3724 (1974).
15. K.-L. Fong, P. B. McCay and J. L. Payer, *J. biol. Chem.* **248**, 7792 (1973).
16. P. J. Catanzaro, H. J. Schwartz and R. C. Graham, *Am. J. Path.* **64**, 387 (1971).
17. M. Di Rosa, J. M. Papadiumitroiou and D. A. Willoughby, *J. Path.* **105**, 239 (1971).
18. M. Di Rosa, J. P. Giroud and D. A. Willoughby, *J. Path.* **104**, 15 (1971).
19. A. Blackham, R. T. Owen, *J. Pharm. Pharmac.* **27**, 201 (1975).
20. R. Vinegar, W. Schreiber and R. Hugo, *J. Pharmac. exp. Ther.* **166**, 96 (1969).
21. A. Nakagawara and S. Minakami, *Biochem. biophys. Res. Commun.* **64**, 760 (1975).
22. B. Halliwell, *Biochem. J.* **135**, 379 (1973).
23. J. S. Bus, S. D. Aust and J. E. Gibson, *Biochem. biophys. Res. Commun.* **58**, 749 (1974).
24. R. J. Mason, T. P. Stossel and M. Vaughan, *J. clin. Invest.* **51**, 2399 (1972).
25. M. N. Jindal, V. V. Kelkar and V. H. Vaishnav, *Br. J. Pharmac.* **52**, 609 (1974).
26. A. L. Willis, *J. Pharm. Pharmac.* **21**, 126 (1969).
27. G. E. Davies, *Immunology* **8**, 291 (1965).
28. J. P. Giroud and D. A. Willoughby, *J. Path.* **101**, 241 (1970).
29. C. Malmsten, M. Hamberg, J. Svensson and B. Samuelsson, *Proc. natn Acad. Sci. USA* **72**, 1446 (1975).
30. G. A. Higgs, E. McCall and J. F. Youlten, *Br. J. Pharmac.* **53**, 539 (1975).
31. S. R. Turner, J. A. Campbell, W. S. Lynn, *J. exp. Med.* **141**, 1437 (1975).
32. F. Lavelle, A. M. Michelson and L. Dimitrijevic, *Biochem. Biophys. Res. Commun.* **55**, 350 (1973).
33. G. Wilhelmi, *Int. Symposium. Nonsteroidal Anti-Inflammatory Drugs, Progs. Proc.* 174 (1965).
34. J. J. Rinehart, S. P. Balcerzak, A. L. Sagone and A. F. LoBuglio, *J. clin. Invest.* **54**, 1337 (1974).
35. G. P. Lewis and P. J. Piper, *Nature* **254**, 308 (1975).
36. R. K. Root, J. Metcalf, N. Oshino and B. Chance, *J. clin. Invest.* **55**, 945 (1974).
37. M. L. Salin and M. McCord, *J. clin. Invest.* **54**, 1005 (1974).