*Br. J. Pharmac.* (1983), **80**, 269–277

# Induction of zymosan-air-pouch inflammation in rats and its characterization with reference to the effects of anticomplementary and anti-inflammatory agents

Satoshi Konno & Susumu Tsurufuji

Department of Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku University, Aoba, Aramaki, Sendai 980, Japan

**1** Induction of an experimental inflammation of the air-pouch type with the aid of zymosan (known to activate the alternative pathway of the complement system) was carried out in an attempt to induce a reproducible inflammatory model suitable for quantitative studies.

**2** Rats were injected subcutaneously with 8 ml of air on the dorsal surface to make an air-pouch, followed 24 h later by 4 ml of 1.6% (w/v) zymosan suspension. This induced inflammatory responses.

**3** Treatment with zymosan suspension provoked exudation of fluid, accumulation of polymorphnuclear leukocytes (PMN) in the pouch and the development of granulation tissue as a wall of the pouch. Approximately half of the PMN in the pouch formed a characteristic layer of aggregated cells sticking onto the inner surface of the pouch wall. They were counted after being completely disaggregated by means of incubation with trypsin.

**4** In preliminary experiments with potential anti-inflammatory drugs, local application of a novel anti-complementary agent, K-76COONa, inhibited leukocyte accumulation in the pouch, whereas the potent anti-inflammatory agent, dexamethasone, was ineffective. By contrast, exudation of the pouch fluid was effectively inhibited by dexamethasone but not by K-76COONa.

## Introduction

Zymosan, a particulate material from the cell wall of yeast, is capable of activating the alternative pathway of the complement system (Pillemer, Blum, Lepow, Ross, Todd & Wardlaw, 1954) and induces secretion of lysosomal enzymes (Weissmann, Dukor & Zurier, 1971; Schorlemmer, Edwards, Davies & Allison, 1977) and release of prostaglandins, leukotriene C and leukotriene $B_4$ (Zurier & Sayadoff, 1975; Doig & Ford-Hutchinson, 1980; Davidson, Smith & Ford-Hutchinson, 1980; Rouzer, Scott, Cohn, Blackburn & Manning, 1980; Claesson, Lundberg & Malmsten, 1981) from polymorphonuclear leukocytes (PMN) and macrophages. Zymosan is also known to induce several types of experimental models of inflammation such as interstitial pneumonitis (Edwards, Wagner & Seal, 1976), hypersensitivity pneumonitis (Barrios, Santos, Figueroa & Reyes, 1980), chronic muscle inflammation (Green & Mangan, 1980), arthritis (Keystone, Schorlemmer, Pope & Allison 1977) and rabbit skin oedema (Williams, 1979; Williams & Jose, 1981). In those models, however, there are some practical differences in making quantitative measurements of the exudative and proliferative components of the inflammatory process, especially in quantifying leukocyte infiltration.

In the present study, an experimental model of inflammation caused by zymosan, designated the zymosan-air-pouch inflammation, was induced in an air-pouch prepared on the dorsum of rats in order to overcome the above-mentioned difficulties. To characterize this inflammation model exudate accumulation, migration of inflammatory cells into the pouch and the amount of granulation tissue formed in the wall of the pouch were measured, together with histological observation of the pouch wall tissue. In addition, the effects of dexamethasone, a steroidal anti-inflammatory drug, and K-76COONa, a specific inhibitor of activation of the fifth component of the complement system (Hong, Kinoshita, Miyazaki, Izawa & Inoue, 1979), on the development of this type of inflammation were examined.

## Methods

### *Animals*

Male rats of the Sprague-Dawley strain (specific pathogen-free, 4 weeks old and weighing 60–80g) were purchased from Charles River Japan, Inc., Kanagawa, Japan, and were maintained on laboratory food (CRF-1, Charles River Japan) and chlorinated tap water in a rack provided with lamina flow of clean air for 2 weeks before use.

### *Materials*

Zymosan A, trypsin (trypsin type III) and dexamethasone were obtained from Sigma Chemical Co., St. Louis, MO., U.S.A. Sodium carboxymethyl cellulose (CMC, Cellogen F-3H, Dai-ichi Kogyo Seiyaku Co., Ni-igata, Japan) and K-76COONa, sodium 6,7-dihydroxy-2,5,5,8a-tetramethyl- 1,2,3, 4,4a,5,6,7,8,8a- decahydronaphthalene-1- spiro-2′-(7′- carboxylate-6′-formyl- 4′-hydroxy-2′, 3′-dihydrobenzofuran), (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan), a specific inhibitor of the fifth component of the complement system (Hong *et al.*, 1979), were generous gifts. Casein (casein nach Hammarsten) was obtained from E. Merck, Darmstadt, W. Germany.

### *Zymosan-air-pouch inflammation*

Rats were injected with 8 ml of air subcutaneously on the back , under light ether anaesthesia, to make an air-pouch in the shape of an ellipsoid or oval. Twenty-four hours later 4 ml of 1.6% (w/v) zymosan suspension in 0.8% (w/v) solution of CMC in 0.9% w/v NaCl solution (saline), supplemented with antibiotics (0.1 mg penicillin G potassium and 0.1 mg dihydrostreptomycin sulphate per 1.0 ml), was injected into the preformed air-pouch to induce an inflammatory response which we call the 'zymosan-air-pouch inflammation'. The above zymosan suspension in the CMC vehicle will be designated 'zymosan-CMC'. To prepare the zymosan-CMC, zymosan particles were washed with about a 30 fold volume of saline, sterilized by autoclaving at 110 °C for 15 min and resuspended at a concentration of 26.7 mg $ml^{-1}$ in sterile saline. Three volumes of the above sterile zymosan suspension and two volumes of 2% (w/v) CMC solution containing NaCl at a concentration of 0.9% (w/v) (autoclaved at 110 °C for 15 min) were mixed; to this antibiotics were then added, as described above, to yield the zymosan-CMC. A 0.8% solution of CMC in saline, autoclaved and containing added antibiotics as described above, was used in the control experiments and is designated 'vehicle-CMC'.

### *Casein-air-pouch inflammation*

The air-pouch was prepared on the back of rats as mentioned above, and 24 h later 4 ml of a solution of 0.8% (w/v) CMC dissolved in saline containing alkali-solubilized casein (ASC) at a concentration of 2% (w/v), and supplemented with antibiotics as for zymosan-CMC, was injected into the preformed air-pouch to induce an inflammatory response which is called the 'casein-air-pouch inflammation'. The above CMC solution containing ASC will be designated 'casein-CMC'. To prepare the casein-CMC, a suitable amount of casein nach Hammarsten was dissolved in distilled water, as described by Wilkinson (1972), by raising the pH to 12 with 1 N NaOH and carefully restoring the solution to neutrality with 1N HCl, to yield a 4% (w/v) solution of alkali-solubilized casein (ASC). One volume of the above 4% ASC solution and one volume of 1.6% (w/v) CMC solution, containing NaCl at a concentration of 1.8% (w/v) (autoclaved at 110 °C for 15 min), were mixed and antibiotics added as described above, to yield the casein-CMC.

### *Measurement of the number of inflammatory cells*

At suitable times after injection of the zymosan-CMC, casein-CMC or vehicle-CMC into the air-pouch, rats were killed by cutting the carotid artery under light ether anaesthesia. Hair on the back was clipped off and the skin was incised at the top of the pouch with scissors to make an opening (0.5–1.0 cm in length) and then the rat was held by hand to keep the abdominal side up and the pouch fluid pressed out carefully from the opening and collected in a plastic cup. The free cells in the pouch fluid were counted in a haemocytometer. As preliminary experiments indicated that the zymosan-air-pouch inflammation was characterized by a heavy aggregation of inflammatory cells sticking to the surface of the inner pouch wall, as shown in Figure 3(b), 0.05% (w/v) trypsin in Hanks' balanced salt solution was infused into the pouch after the collection of the pouch fluid and then the dead rat was kept at 37 °C for 4 h in an incubator in order to disaggregate those cells sticking onto the pouch wall. The disaggregated cells were counted in a haemocytometer immediately after finishing the trypsinization. Although no significant aggregation of inflammatory cells on the pouch wall was observed in the histological specimens of casein-air-pouch inflammation (data not shown), the same trypsinization procedure was followed in order to make the cell counts comparable between the two types of air-pouch inflammation.

*Measurement of the wet weight of granulation tissue*

Granulation tissue which was formed as a wall of the pouch was carefully excised from surrounding loose connective tissues and weighed.

*Histological examinations*

At appropriate times after the injection of the zymosan-CMC, pouch wall tissues were excised together with the dorsal skin, fixed immediately in phosphate buffered (pH 7.0) 3.7% formaldehyde solution for 48 h, embedded in paraffin, sectioned at 2μm width and stained with haematoxylin and eosin.

*Drug treatment*

Dexamethasone dissolved in 0.1 ml of 99.5% ethanol was injected directly into each pouch immediately after the injection of 4 ml of the zymosan-CMC or casein-CMC into the preformed air-pouch. The injection of 0.1 ml of 99.5% ethanol into the pouch had been shown, in preliminary experiments, not to affect the course of the inflammatory response in this model. When K-76COONa was administered, it was dissolved in distilled water by raising the pH to 7.4 with 0.1N NaOH in order to neutralize the phenolic hydroxy group of the molecule, and the resultant solution of K-76COONa was then diluted to the desired concentration, made isotonic by adding NaCl and used in place of saline to prepare the zymosan suspension according to the procedure described already.

## Results

*Fluid exudation in the pouch*

When the zymosan-CMC was injected to induce inflammation in a preformed air-pouch, the volume of the pouch fluid increased slowly during the first 48 h. Thereafter accumulation of the pouch fluid intensified as indicated in Figure 1. On the other hand, when the vehicle-CMC was injected as control, the volume of the pouch fluid decreased gradually with the passage of time (Figure 1), and at day 5 there was only a trace of viscous fluid. Twenty-four hours after the initiation of the zymosan-air-pouch inflammation, the volume of pouch fluid reached a level about twice as high as that in the control rats which were injected with the vehicle-CMC; the difference between the two groups increased to about three fold at 48 h.

*Inflammatory cells in the pouch*

In the pouch injected with zymosan-CMC, a large number of inflammatory cells were found to be aggregated and sticking onto the inner surface of the pouch wall (Figure 3b), while there were many free

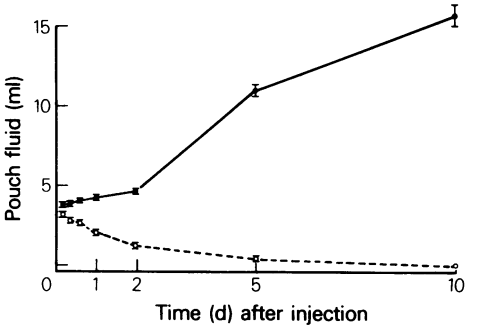


**Figure 1** Time course of pouch fluid accumulation in the zymosan-air-pouch inflammation. Each 4ml of the zymosan-CMC (1.6% zymosan suspension in 0.8% CMC solution in 0.9% NaCl, ●), and the vehicle-CMC (0.8% CMC solution in 0.9% NaCl, ○), was injected into a preformed air-pouch. Each point represents the mean ± s.e. mean from 7 animals.

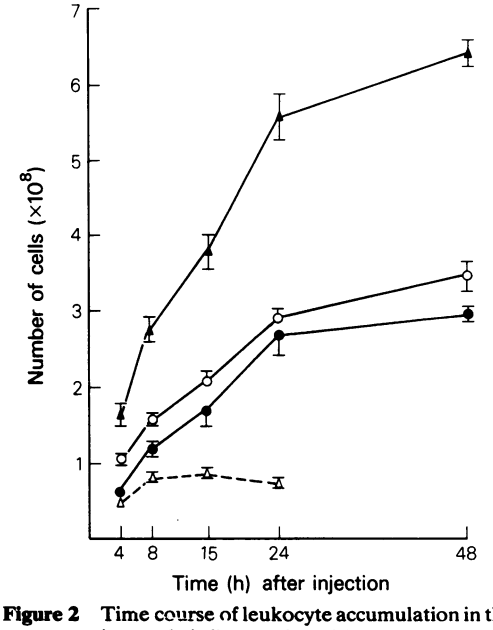


**Figure 2** Time course of leukocyte accumulation in the zymosan-air-pouch inflammation. The data are taken from the same experiment as that shown in Figure 1. In the zymosan-CMC group the numbers of inflammatory cells free in the pouch fluid (●) and those disaggregated from the pouch wall by trypsinization (○) were counted and the sum total of the above two elements was calculated and shown as total cells (▲). In the vehicle-CMC group, only inflammatory cells free in the pouch fluid (△) were counted since there were no cells aggregated on the surface of the pouch wall. Each point represents the mean ± s.e. mean, $n = 7$.

cells in the pouch fluid as well. The adherent cells were disaggregated by trypsinization and then counted. In preliminary experiments we confirmed that the typsinization procedure did not adversely affect the measurement of cell numbers: there was no difference in cell counts performed before and after trypsinization in exudate cells collected from rats 15 h after i.p. injection of 1% (w/v) casein in Krebs-Ringer bicarbonate solution. Figure 2 shows that the number of disaggregated and free cells in the zymosan-CMC-treated pouch increased quickly during the first 24 h but much less rapidly during the second 24 h.

In the pouch injected with the vehicle-CMC, no aggregation of inflammatory cells on the pouch wall was detectable after histological examination (Figure 3a), so only free cells were counted in the pouch fluid in this group. The time course of accumulation of these free inflammatory cells in the pouch is shown in Figure 2.

Microscopic study of smears of the cells collected from the pouch fluids revealed that in the zymosan-CMC group, more than 95% of the cells were polymorphonuclear leukocytes (PMN) throughout the period from 4 h till 48 h. Most of these cells had phagocytosed zymosan particles. Even in the specimens taken from 5-day pouch fluids, 90% of the cells were still PMN whereas the rest were macrophages engulfing zymosan particles and a basophilic amorphous substance which was thought to be CMC residue. On the other hand, in the vehicle-CMC group, 40% of the cells were macrophages in 24 h samples, while 80% of the cells in specimens collected from the pouch fluid not later than 8 h were PMN.

Figure 2 illustrates the marked differences between the zymosan-CMC and vehicle-CMC groups, throughout the experimental periods, in the rate of migration of the inflammatory cells into the pouch. At 4 h the total number of cells in the zymosan-CMC group was more than three times as much as that of the vehicle-CMC group. Thereafter, the differences was further augmented with the passage of time.

*Granulation tissue*

In the zymosan-CMC group, distinct granulation tissue was formed as a wall of the pouch and by day 5 it became quite tough enough to be easily separable from the surrounding subcutaneous tissues. The wet weight of the granulation tissue isolated was measured after removing a layer of the inflammatory cells aggregated on the inner surface of the pouch. However, in the vehicle-CMC group the granulation tissue was jelly-like and fragile. As reflected by measurement of the wet weight of the granulation tissues (Table 1), there was a distinct difference in the proliferative process betweeen the zymosan-CMC and vehicle-CMC groups.

*Histological observation*

Twenty-four hours after the injection of the vehicle-CMC subcutaneous tissues surrounding the pouch were only slightly oedematous and infiltration of a small number of inflammatory cells consisting mainly of mononuclear cells accompanied by a few PMN were observed (Figure 3a). In contrast, considerable oedematous swelling of the subcutaneous tissues and moderate infiltration of PMN and mononuclear cells was observed in the zymosan-CMC group (Figure 3b). A characteristic feature of the zymosan-CMC group is the formation of a prominent layer of aggregated PMN sticking onto the inner surface of the pouch wall. This layer of aggregated cells could be completely removed by incubating the tissues at 37 °C for 4 h with 0.05% trypsin in Hanks' balanced salt solution (figure 3c). On day 5, heavy deposition of a basophilic amorphous foreign substance (thought to be residual CMC) was observed in the pouch wall subcutaneous tissues of the vehicle-CMC group (Figure 4a), whilst in the zymosan-CMC group massive proliferation of granulation tissues was observed (Figure 4b) together with a small amount of the basophilic amorphous foreign substance in the granulation tissues.

**Table 1** Formation of capsular granulation tissues in zymosan-air-pouch inflammation

| *Days after injection* | *Group* | *Granulation tissues wet weight* (g) | P |
|---|---|---|---|
| 5 | Vehicle-CMC | 1.02 ± 0.04 | <0.001 |
| | Zymosan-CMC | 3.73 ± 0.14 | |
| 10 | Vehicle-CMC | 0.99 ± 0.04 | <0.001 |
| | Zymosan-CMC | 3.80 ± 0.13 | |

Rats were prepared with zymosan-air-pouch inflammation by injecting zymosan-CMC or vehicle-CMC (for controls) as described in Methods; the granulation tissue was weighed 5 or 10 days later.

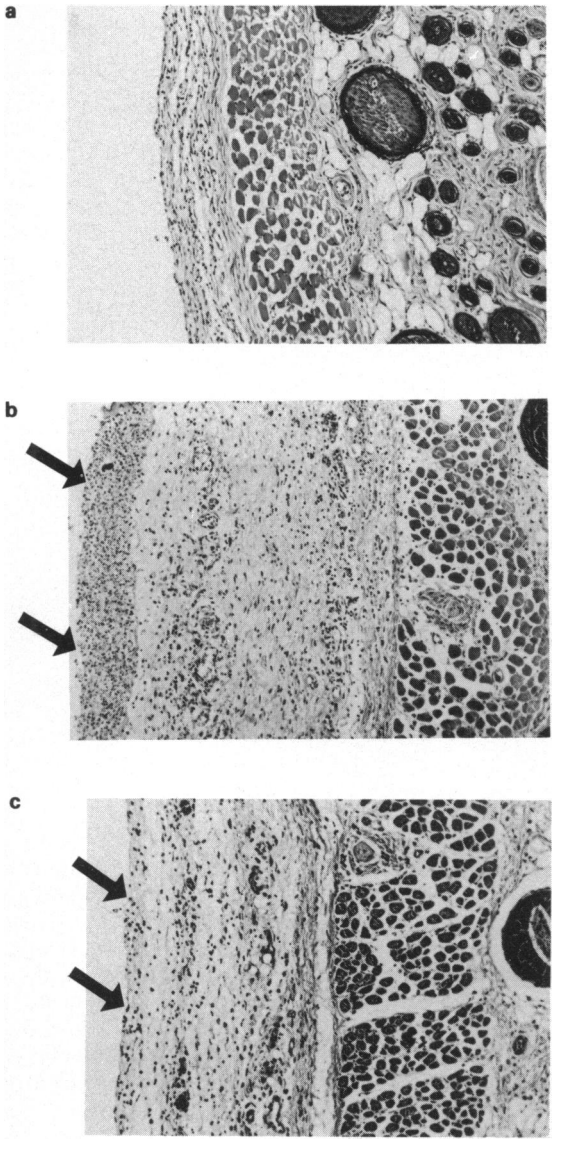


**Figure 3** Histology of the pouch wall subcutaneous tissues. The specimens were taken 24 h after the injection of zymosan-CMC or vehicle-CMC into the preformed air-pouch. (Haematoxylin and eosin stain, × 100) (a) Vehicle-CMC group; slight oedematous change in the subcutaneous tissue and no aggregation of inflammatory cells on the surface of the pouch wall. (b) Zymosan-CMC group; highly oedematous swelling of the subcutaneous tissue and heavy aggregation of polymorphonuclear leukocytes (PMN) on the surface of the pouch wall subcutaneous tissue, indicated by the arrows. (c) Zymosan-CMC group after trypsinization; the layer of the aggregated PMN is eliminated (arrows) without damaging the pouch wall subcutaneous tissue.

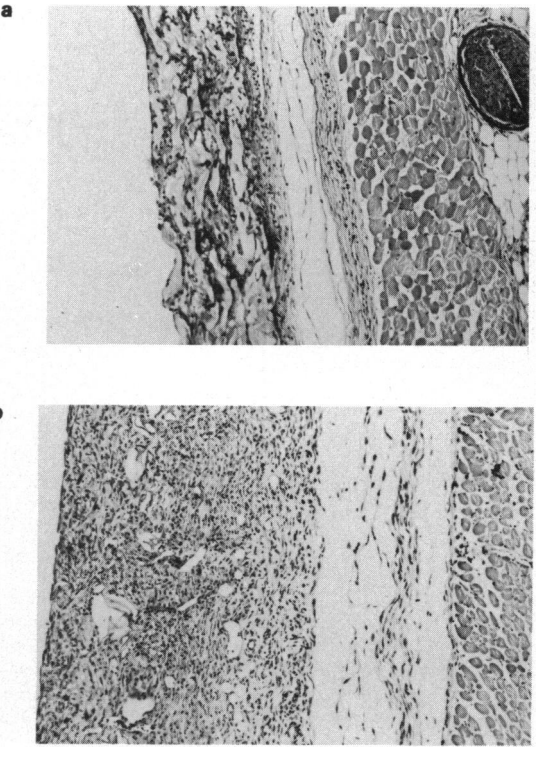


**Figure 4.** Histology of the pouch wall granulation tissues. The specimens were taken 5 days after the injection of zymosan-CMC or vehicle-CMC. (Haematoxylin and eosin stain, × 100) (a) Vehicle-CMC group; heavy deposition of amorphous foreign substance in the pouch wall subcutaneous tissue. (b) Zymosan-CMC group; well developed granulation tissue as a wall of the pouch but only slight deposition of amorphous foreign substance.

### *Effect of local application of K-76COONa on zymosan-air-pouch inflammation*

Zymosan is well known as an agent capable of activating the alternative pathway of the complement system (Pillemer *et al.*, 1954). Assuming that complement activation plays an important role in the development of the zymosan-air-pouch inflammation, we attempted to investigate whether K-76COONa, a specific inhibitor of the activation of the fifth component of complement (Hong *et al.*, 1979), could inhibit the development of the zymosan-air-pouch inflammation. K-76COONa was injected into the air-pouch after being mixed with the zymosan-CMC; 6 h later the rats were killed. The results summarized in Figure 5 show the effects of locally injected K-76COONa on both the leukocyte and pouch fluid accumulation in the period of the first

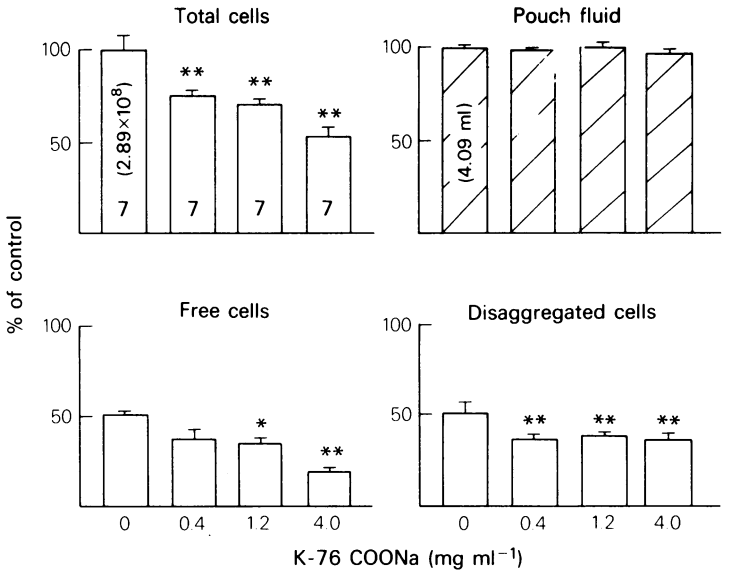


**Figure 5** The effect of local application of K-76COONa on the zymosan-air-pouch inflammation. At 6 h after the injection of the zymosan-CMC containing various amounts of K-76COONa, the volume of the pouch fluid and the number of the cells in the pouch were assayed. Each column represents the mean ± s.e. mean. Figures in the columns indicate the number of rats used. Figures in parentheses show the number of inflammatory cells and the volume of the pouch fluid in the control group. Statistically significant differences with respect to the corresponding control are shown by the following symbols: $*P<0.025$, $**P<0.01$.

6 h. K-76COONa at doses ranging from 1.6 to 16 mg per pouch, i.e. giving an initial concentration in the pouch fluid of 0.4–4.0 mg ml$^{-1}$, significantly suppressed the number of both free and disaggregated cells but did not affect the pouch fluid accumulation.

*Effect of local application of dexamethasone on zymosan-air-pouch inflammation*

One possible mechanism for the anti-inflammatory action of glucocorticoids is an indirect inhibitory effect on the generation of proinflammatory metabolites of arachidonic acid via a peptide-mediated inhibitory action on phospholipase $A_2$ (Hong & Levine, 1976; Bray & Gordon, 1978; Blackwell, Flower, Nijkamp & Vane, 1978; Robinson, McGuire, Bastian, Kantrowitz & Levine, 1978; Blackwell, Carnuccio, Di Rosa, Flower, Parente & Persico, 1980; Hirata, Schiffmann, Venkatasubramanian, Salomon & Axelrod, 1980). With reference to the above concept, the effect of dexamethasone on the zymosan-air-pouch inflammation was examined in an attempt to collect further data on the anti-inflammatory action of this steroid. Dexamethasone was administered locally into the pouch immediately after the injection of the zymosan-CMC, and animals were killed 6 h later. The results are summarized in Figure 6. In contrast to the results from the experiment with K-76COONa, dexamethasone exerted a dose-dependent suppressive effect on pouch fluid accumulation, without altering the total number of leukocytes emigrating into the pouch fluid. However, although dexamethasone did not alter the overall number of leukocytes in the pouch, it significantly affected their distribution between free and aggregated states in a dose-dependent manner (Figure 6).

*Effect of local application of dexamethasone on casein-air-pouch inflammation*

As the local application of dexamethasone in the zymosan-air-pouch inflammation did not inhibit leukocyte accumulation at the inflammatory locus (Figure 6), a similar experiment was designed with alkali-solubilized casein used in place of zymosan as a chemoattractant for leukocytes (Wilkinson, 1972). Using the same experimental design we found that accumulation of pouch fluid and migration of leukocytes into the pouch were both markedly inhibited in a dose-dependent manner by dexamethasone (Figure 7).

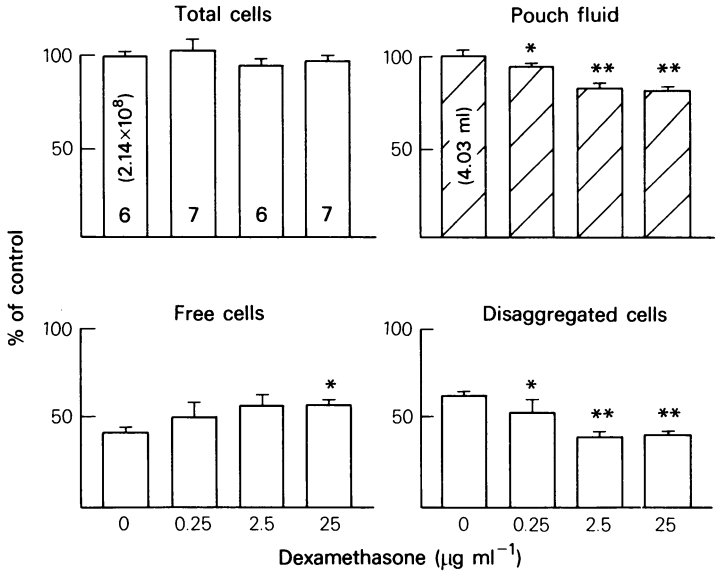


**Figure 6** The effect of local application of dexamethasone on the zymosan-air-pouch inflammation. Immediately after the injection of the zymosan-CMC into the preformed air-pouch, a suitable amount of dexamethasone dissolved in 99.5% ethanol (0.1 ml per rat) was injected locally so as to achieve the designated concentration in the pouch fluid. The assays were done 6 h later. Each column represents the mean ± s.e. mean. Figures in the columns indicate the number of rats used. Figures in parentheses show the number of inflammatory cells and volume of the pouch fluid in the control group. Statistically significant differences with respect to the corresponding control are shown by the following symbols: $*P<0.05$, $**P<0.01$.

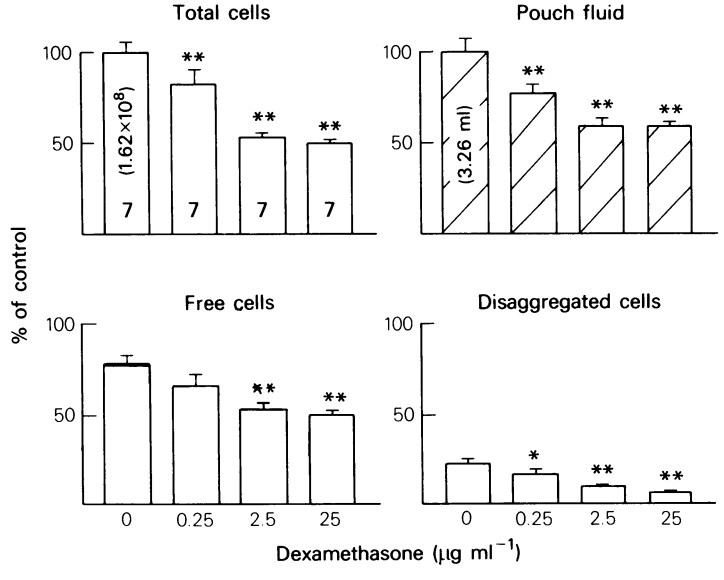


**Figure 7** The effect of local application of dexamethasone on the casein-air-pouch inflammation. Immediately after the injection of the casein-CMC into the preformed air-pouch, a suitable amount of dexamethasone dissolved in 99.5% ethanol (0.1 ml per rat) was injected locally so as to achieve the designated concentration in the pouch fluid. The assays were done 6 h later. Each column represents the mean ± s.e. mean. Figures in the columns indicate the number of rats used. Figures in parentheses show the number of inflammatory cells and volume of the pouch fluid in the control group. Statistically significant differences with respect to the corresponding control are shown by the following symbols: $*P<0.05$, $**P<0.01$.

## Discussion

The air-pouch method for the induction of inflammation models was first introduced by Selye with the aid of croton oil as a phlogistic agent (Selye, 1953). We have developed various modifications of his original method by introducing a variety of characteristic stimuli involving simple foreign substances such as carrageenan (Fukuhara & Tsurufuji, 1969; Tsurufuji, Sato, Min & Ohuchi, 1978), carboxymethyl cellulose (Ishikawa, Mori & Tsurufuji, 1968) and more complex allergic mechanisms (Tsurufuji, Yoshino & Ohuchi, 1982; Ohuchi, Yoshino, Kanaoka, Tsurufuji & Levine, 1982). In the course of those studies it became evident that the air-pouch method has a definite advantage in supplying a suitable space for the induction of inflammatory responses (Tsurufuji *et al.*, 1982). Based on those experiences the present experiment was designed to develop an inflammatory model of the air-pouch type with the aid of a complement activator, i.e. zymosan. Infusion into the preformed air-pouch of zymosan suspended in a viscous solution of CMC provoked inflammatory responses involving exudation of fluid, migration of inflammatory cells (predominantly polymorphonuclear leukocytes), development of a granuloma pouch and formation of a characteristic layer of aggregated polymorphonuclear leukocytes (PMN) sticking together with zymosan particles onto the inner surface of the inflammatory pouch. Since zymosan provokes activation of the alternative pathway of the complement system (Pillemer *et al.*, 1954), it is likely that immune adherence of PMN with C3b-coated zymosan particles is responsible for the formation of the aggregated layer of leukocytes.

Complement-derived chemotactic factor(s), especially C5a and/or C5a des Arg (Borel & Feurer, 1978; Webster, Hong, Johnston & Henson, 1980), seem to play an important role in the accumulation of PMN in the pouch, since K-76COONa, a specific inhibitor of the activation of C5 (Hong *et al.*, 1979), reduced the number of leukocytes in the zymosan-air-pouch inflammation (Figure 5), whereas this drug failed to affect leukocyte accumulation in the casein-air-pouch inflammation (data not shown), in which alkali-solubilized casein, known as a potent chemoattractant (Wilkinson, 1972), was used instead of zymosan. This result also suggests that anticomplementary agents might be effective therapeutic agents in some inflammatory and allergic diseases. However, in spite of the inhibition of leukocyte emigration by K-76COONa, fluid exudation in the pouch was not inhibited by this agent. Further investigation is needed to elucidate the mechanisms for this different effect of K-76COONa.

In contrast with K-76COONa, the potent anti-inflammatory steroid, dexamethasone, did not inhibit leukocyte migration in spite of significantly suppressing the fluid exudation (Figure 6). Potent inhibitory effects of glucocorticoids on leukocyte accumulation and plasma exudation at the inflammatory locus have been frequently reported in the literature (e.g. Ward, 1966; Ishikawa *et al.*, 1968; 1969; Higgs, Flower & Vane, 1979; Ohuchi *et al.*, 1982). In agreement with this general concept, dexamethasone exerted potent inhibitory effects not only on fluid exudation but also on leukocyte accumulation in the casein-air-pouch inflammation (Figure 7). Dose levels, time and route of administration of dexamethasone in this latter casein-air-pouch experiment were the same as for the zymosan-air-pouch inflammation experiment. Therefore, the failure of dexamethasone to inhibit leukocyte migration in the zymosan-air-pouch inflammation appears to be a peculiar case in respect to the mode of action of the anti-inflammatory steroid. As a matter of fact, leukocyte migration *in vivo* towards complement-derived chemoattractant(s) might be refractory to the anti-inflammatory action of glucocorticoids. Further investigation is required to elucidate these questions.

In summary, this new experimental model appears to be useful for quantitative measurements and biochemical analysis of the inflammatory process in which complement activation plays an important role because inflammatory exudate in the pouch, inflammatory cells free in the pouch fluid as well as those aggregated on the inner surface of the pouch walls and granulation tissues may all be easily collected quantitatively.

### References

BARRIOS, R., SANTOS, G.G., FIGUEROA, J. & REYES, P.A. (1980). Zymosan-induced experimental hypersensitivity pneumonitis in rabbits. *Am. J. Pathol.*, **99**, 731–740.

BLACKWELL, G.J., CARNUCCIO, R., DI ROSA, M., FLOWER, R.J., PARENTE, L. & PERSICO, P. (1980). Macrocortin: a polypeptide causing the anti-phospholipase effect of glucocorticoids. *Nature* **287**, 147–149.

BLACKWELL, G.J., FLOWER, R.J., NIJKAMP, F.P. & VANE, J.R. (1978). Phospholipase $A_2$ activity of guinea-pig isolated perfused lungs. *Br. J. Pharmac.*, **62**, 79–89.

BOREL, J.F. & FEURER, C. (1978). In vivo effects of anti-inflammatory and other drugs on granulocyte emigration in the rabbit skin collection chamber. *J. Path.*, **124**, 85–93.

BRAY, M.A. & GORDON, D. (1978). Prostaglandin production by macrophages and the effect of anti-inflammatory drugs. *Br. J. Pharmac.*, **63**, 635–642.

CLAESSON, H., LUNDBERG, U. & MALMSTEN, C. (1981). Serum-coated zymosan stimulates the synthesis of leukotriene $B_4$ in human polymorphonuclear leukocytes. Inhibition by cyclic AMP. *Biochem. Biophys. Res. Commun.*, **99,** 1230–1237.

DAVIDSON, E.M., SMITH, M.J.H. & FORD-HUTCHINSON, A.W. (1980). Prostaglandin and thromboxane production by rat macrophages. In *Inflammation; Mechanisms and Treatment.* Abstr., ed. Willoughby, D.A. & Giroud, J.P. pp. 301, Lancaster, England: MTP Press Limited.

DOIG, M.V. & FORD-HUTCHINSON, A.W. (1980). Prostaglandin and thromboxane production by rat macrophages. In *Inflammation: Mechanisms and Treatment.* Abstr., ed. Willoughby, D.A. & Giroud, J.P. pp. 303. Lancaster, England: MTP Press Limited.

EDWARDS, J.H., WAGNER, J.C. & SEAL, R.M.E. (1976). Pulmonary responses to particulate materials capable of activating the alternative pathway of complement. *Clin. Allergy*, **6,** 155–164.

FUKUHARA, M. & TSURUFUJI, S. (1969). The effect of locally injected anti-inflammatory drugs on the carrageenin granuloma in rats. *Biochem. Pharmac.*, **18,** 475–484.

GREEN, A.P. & MANGAN, F.R. (1980). The effect of steroidal and non-steroidal anti-inflammatory drugs on chronic muscle inflammation. *J. Pharm. Pharmac.*, **32,** 319–322.

HIGGS, G. A., FLOWER, G. J. & VANE, J. R. (1979). A new approach to anti-inflammatory drugs. *Biochem. Pharmac.*, **28,** 1959–1961.

HIRATA, F., SHIFFMANN, E., VENKATASUBRAMANIAN, K., SALOMON, D. & AXELROD, J. (1980). A phospholipase $A_2$ inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proc. natn. Acad. Sci. USA.*, **77,** 2533–2536.

HONG, K., KINOSHITA, T., MIYAZAKI, W., IZAWA, T. & INOUE, K. (1979). An anticomplementary agent, K-76 monocarboxylic acid: its site and mechanism of inhibition of the complement activation cascade. *J. Immunol.*, **122,** 2418–2423.

HONG, S.L. & LEVINE, L. (1976). Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. *Proc. natn. Acad. Sci. U.S.A.*, **73,** 1730–1734.

ISHIKAWA, H., MORI, Y. & TSURUFUJI, S. (1968). The inhibitory effect of some steroidal anti-inflammatory agents on leucocyte emigration by the carboxymethyl cellulose pouch method. *J. Pharmac. Soc. Jap.*, **88,** 1491–1493.

ISHIKAWA, H., MORI, Y. & TSURUFUJI, S. (1969). The characteristic feature of glucorticoids after local application with reference to leucocyte migration and protein exudation. *Eur. J. Pharmac.*, **7,** 201–205.

KEYSTONE, E.C., SCHORLEMMER, H.U., POPE, C. & ALLISON, A.C. (1977). Zymosan-induced arthritis: A model of chronic proliferative arthritis following activation of the alternative pathway of complement. *Arthritis Rheum.*, **20,** 1396–1401.

OHUCHI, K., YOSHINO, S., KANAOKA, K., TSURUFUJI, S. & LEVINE, L. (1982). A possible role of arachidonate metabolism in allergic air pouch inflammation in rats. Anti-inflammatory effect of indomethacin and dexamethasone and the level of prostaglandin $E_2$ in the exudate. *Int. Archs. Allergy appl. Immunol.*, **68,** 326–331.

PILLEMER, L., BLUM, L. LEPOW, I.H., ROSS, O.A., TODD, E.W. & WARDLAW, A.C. (1954). The properdin system and immunity: I. Demonstration and isolation of a new serum protein, properdin, and its role in immune phenomena. *Science*, **120,** 279–285.

ROBINSON, D.R., McGUIRE, M.B., BASTIAN, D., KANTROWITZ, F. & LEVINE, L. (1978). The effects of anti-inflammatory drugs on prostaglandin production by rheumatoid synovial tissue. *Prostaglandins Med.*, **1,** 461–477.

ROUZER, C.A., SCOTT, W.A., COHN, Z.A., BLACKBURN, P. & MANNING, J.M. (1980). Mouse peritoneal macrophages release leukotriene C in response to a phagocytic stimulus. *Proc. natn. Acad. Sci. U.S.A.*, **77,** 4928–4932.

SCHORLEMMER, H.U., EDWARDS, J.H., DAVIES, P. & ALLISON, A.C. (1977). Macrophage responses to mouldy hay dust, Micropolyspora faeni and zymosan, activators of complement by the alternative pathway. *Clin. exp. Immunol.*, **27,** 198–207.

SELYE, H. (1953). Use of "granuloma pouch" technic in the study of anti-phlogistic conticoids. *Proc. Soc. exp. Biol. Med.*, **82,** 328–333.

TSURUFUJI, S., SATO, H., MIN, K.R. & OHUCHI, K. (1978). Difference in the anti-inflammatory effect of indomethacin between acute and chronic stages of carrageenin-induced inflammation. *J. Pharm. Dyn.*, **1,** 8–14.

TSURUFUJI, S., YOSHINO, S. & OHUCHI, K. (1982). Induction of an allergic air-pouch inflammation in rats. *Int. Arch. Allergy appl. Immunol.*, **69,** 189–198.

WARD, P.A. (1966). The chemosuppression of chemotaxis. *J. exp. Med.*, **124,** 209–229.

WEBSTER, R.O., HONG, S.R., JOHNSTON, R.B., JR. & HENSON, P.M. (1980). Biological effects of the human complement fragments C5a and C5a des Arg on neutrophil function. *Immunopharmacology*, **2,** 201–219.

WEISSMANN, G. DUKOR, P. & ZURIER, R.B. (1971). Effect of cyclic AMP on release of lysosomal enzymes from phagocytes. *Nature New Biol.*, **231,** 131–135.

WILKINSON, P.C. (1972). Characterization of the chemotactic activity of casein for neutrophil leucocytes and macrophages. *Experientia*, **28,** 1051–1052.

WILLIAMS, T.J. (1979). Prostaglandin $E_2$, prostaglandin $I_2$ and vascular changes of inflammation. *Br. J. Pharmac.*, **65,** 517–524.

WILLIAMS, T.J. & JOSE, P.J. (1981) Mediation of increased vascular permeability after complement activation. *J. exp. Med.*, **153,** 136–153.

ZURIER, R.B. & SAYADOFF, D.M. (1975). Release of prostaglandins from human polymorphonuclear leukocytes. *Inflammation*, **1,** 93–101.

*(Received April 15, 1983. Revised June 10, 1983.)*