*Br. J. Pharmac.* (1971), **42**, 392–402.

# Mediators of the inflammation induced in the rat paw by carrageenin

PEARL CRUNKHORN AND S. C. R. MEACOCK

*Lilly Research Centre Limited, Erl Wood Manor, Windlesham, Surrey*

**Summary**

1. The time course of oedema formation in rats caused by injection of carrageenin into the paw was followed for 5·5 hours. Intact or adrenalectomized rats which had previously been injected with ellagic acid or saliva to reduce considerably the concentration of blood kininogens, or with methysergide to antagonize 5-hydroxytryptamine (5-HT) showed a reduced inflammatory response. It was concluded that kinins and 5-HT contributed significantly to oedema formation during this period.

2. Mepyramine alone had no effect on oedema formation, but in combination with ellagic acid treatment, with or without methysergide, it caused a reduction suggesting that histamine played a minor role in oedema formation during the first 3 hours.

3. Vascular permeability studies indicated that injection of ellagic acid did not interfere with the normal responses in skin to intradermal injections of histamine, 5-HT, bradykinin or compound 48/80. Mepyramine and methysergide, at the doses used in the carrageenin experiments, completely antagonized histamine and 5-HT, respectively, and did not affect the skin responses of bradykinin.

4. Treatment *in vivo* with ellagic acid or rat saliva was equally effective in reducing plasma kininogen concentrations by an amount equivalent to more than 10 times the quantity of substrate 1 measured by Gautvik & Rugstad (1967).

5. Rat saliva, but not ellagic acid, lowered complement levels by approximately 20%.

## Introduction

The inflammatory response to an injection of carrageenin into the rat paw was introduced by Winter, Risley & Nuss (1962) and developed by these workers (Winter, Risley & Nuss, 1963 ; Winter, 1965) as a model of inflammation in the search for anti-inflammatory drugs. The compounds used in these studies were chosen for their ability to suppress oedema rather than for specific antagonist activity, and therefore the role and importance of the various mediators released during the inflammatory reaction have yet to be clearly defined.

Recently, Di Rosa & Sorrentino (1968, 1970) have shown both by inhibition of plasma kallikrein with aprotinin and by depletion of plasma kininogen with cellulose sulphate, that kinins are released in the inflammatory response to carrageenin in the

rat. The kininogen concentrations of female Wistar rats were greatly reduced by intravenous injection of ellagic acid (Gautvik & Rugstad, 1967) or rat saliva and these procedures have been used in our investigation. The extent of the contribution to the oedema formation made by 5-hydroxytryptamine (5-HT) is indicated in the studies of Fekete & Kürti (1970) in which methysergide, an antagonist of 5-HT, was found to suppress the carrageenin reaction. In this study we have observed changes in the time course of oedema formation, in animals having low concentrations of plasma kininogen as well as in animals treated with antagonists of histamine and 5-HT in an attempt to determine the relative importance of kinins, histamine and 5-HT in the production of oedema by carrageenin.

## Methods

### *Carrageenin inflammation*

Female Wistar rats (140–160 g), maintained on unrestricted supplies of food and water, were used. While injections were being given the animals were lightly anaesthetized with ether except in one series of experiments as described in **Results** when they were anaesthetized with pentobarbitone sodium (Abbot), (40 mg/kg i.p.) 30 min before treatment.

Ellagic acid (Koch-Light Laboratories) was used in a standard solution of $2 \times 10^{-4}$M in a 0·15 M Tris-HCl buffer, pH 7·35 (Gautvik & Rugstad, 1967). Methysergide bimaleate (Sandoz) and mepyramine maleate (May and Baker) were dissolved in 0·9% NaCl. Rat saliva was collected during pentobarbitone anaesthesia after stimulation of salivation by an intraperitoneal injection of pilocarpine (2 mg/kg). It was then centrifuged and stored at −26° C until used.

Inflammation was induced by injecting 0·1 ml of 1% homogenized suspension of carrageenin (Gelozone ST1, Whiffen, Loughborough) in 0·9% NaCl into the plantar surface of the right hind paw of each rat. The volume of the inflamed paw was determined using a mercury plethysmograph (U. Basile, Milan). Measurements of the right hind paw were made after 1 h, 2·5 h, 3·5 h, 4·5 h and 5·5 h and the amount of oedema was determined by comparison with the volume of the left hind paw measured at 1 hour. At least five readings were taken for each paw at any one time. Ellagic acid solution (0·5 ml) or rat saliva (0·5 ml) was injected slowly into a tail vein, 3 times at 5 min intervals, 30 min before the injection of carrageenin. In those experiments where both reagents were injected, a 15 min interval was allowed between the injections of ellagic acid and saliva. Mepyramine and methysergide, both at doses of 2·5 mg base/kg, were injected intravenously 15 min before the carrageenin injection. Further injections of methysergide were given 2 h and 4 h after the carrageenin injection to maintain antagonism to 5-HT. In some experiments methysergide and/or mepyramine were given 15 min after ellagic acid.

Bilateral adrenalectomy was performed through two dorsolateral incisions while under pentobarbitone anaesthesia. Each animal was injected intraperitoneally with 0·5 ml bemigride (Nicholas) to hasten recovery from anaesthesia, and maintained with 0·45% NaCl in the drinking water. Only those animals in a lively healthy condition were used 4 days later.

### *Increased vascular permeability*

Rats were anaesthetized with methohexitone sodium (Lilly, 40 mg/kg) and their abdomens were shaved. They were then injected intravenously with solutions of

5% Pontamine Sky Blue dye (Gurr, 0·2 ml/100 g) and mepyramine or methysergide. Five minutes later intradermal injections were made in the abdominal skin using serial dilutions of either histamine (British Drug Houses), 5-HT (Sandoz), or synthetic bradykinin (Sandoz) in 0·1 ml of Tyrode solution, or with compound 48/80 in 0·9% NaCl adjusted to pH 7·3. Forty-five minutes later the animals were killed by cervical dislocation. The inflammatory response was determined by measuring the mean diameters of the blue lesions of the reflected skin and by assessing the dye intensity on an arbitrary 0–10 point scale. Rats previously treated with ellagic acid were not subjected to skin reactions until 30 min after ellagic acid treatment, to allow blood pressure to return to normal (Gautvik & Rugstad, 1967).

### *Kininogen concentrations*

Kininogen concentrations were measured using denatured whole blood as substrate according to the method of Brocklehurst & Zeitlin (1967), with the following slight modifications. The denatured substrate was homogenized in 0·02 M sodium phosphate buffer, pH 7·35. Aliquots (1 ml) of this suspension, containing substrate from 0·3 ml of fresh blood, were incubated at 37° C for 30 min with excess crystalline trypsin (Tryptar, Armour, 1–3 mg, as previously determined for each batch of enzyme) in 4 ml of phosphate buffer. The released kinin was measured on the isolated guinea-pig ileum bathed in oxygenated Tyrode solution at 37° C, using synthetic bradykinin as a standard. Disposable polystyrene syringes and siliconized needles and glassware were used in the collection of all blood samples, the extraction process, and the assay of kinin.

### *Total haemolytic complement determinations*

Serum concentrations of total haemolytic complement were measured by the method of Rosenberg & Tachibana (1962) except that double quantities of each reagent were used and the degree of erythrocyte haemolysis was determined from the optical density reading of the erythrocyte supernatant at 541 mμ with a Unicam SP600 spectrophotometer. Complement titres were expressed in terms of C'H50 units/ml, where one C'H50 unit is defined as the amount of complement in that dilution of serum which caused 50% lysis of the sensitized erythrocytes. Blood samples were taken from the tail or by cardiac puncture. The sera were harvested within 2 h, quick-frozen and stored at −26° C until used. Statistical analysis was by Student's *t* test.

## Results

### *Variation in animal response to the injection of carrageenin*

The time course of oedema formation in the paws of ten female rats is shown in Fig. 1 and is typical of the variation in animal behaviour seen throughout this investigation. Experiments using male animals (unpublished observations), adrenalectomized female animals and animals treated with antagonists of possible mediators provided data with similar variations within each group, as did experiments using any one type of animal on the same or different days. In order to increase the significance of results, therefore, large groups of animals of the same sex and strain had to be used.

*Effect of ellagic acid, saliva and methysergide*

Rats were treated with ellagic acid, saliva or methysergide and the time course of formation of the oedema caused by carrageenin was followed. All three treatments suppressed oedema by 40–60% during the first 5·5 h, indicating a significant contribution by kinins and 5-HT to oedema formation during this time period. The results which are illustrated in Fig. 2 also indicate that methysergide or saliva were more effective than ellagic acid. Treatment with ellagic acid and saliva together was more effective in the first 3 h than treatment with each separately ($P<0·01$ at 2·5 h). In Fig. 3 it can be seen that ellagic acid and methysergide suppressed carrageenin oedema in adrenalectomized animals and therefore did not act indirectly by adrenal stimulation.

The time course of oedema formation in animals treated with ellagic acid, mepyramine and methysergide in various combinations is given in Table 1. Animals previously anaesthetized with pentobarbitone were used in these experiments. Treatment with ellagic acid or methysergide alone produced a significant depression in oedema formation (45% ; $P<0·001$). Ellagic acid and methysergide together

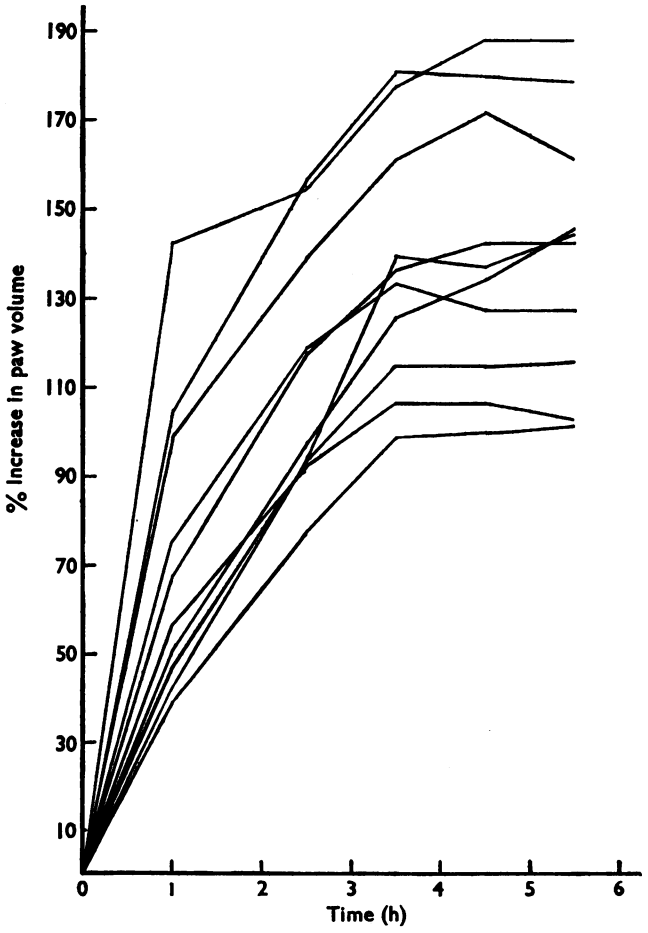


FIG. 1. Time course of oedema formation induced by carrageenin in ten female Wistar rats.

appeared to be more effective than either compound alone but the increased suppression was statistically significant only after 2·5 h ($P<0·05$). Mepyramine was ineffective alone. However, when combined with ellagic acid treatment (but not with methysergide) or with both ellagic acid and methysergide a significant contribution to the suppression of oedema formation was observed during the first 3·5 hours.

*Specificity of treatment with ellagic acid, methysergide and mepyramine*

The results in Table 2 indicate that ellagic acid did not affect the size of the skin lesions caused by intradermal injection of 5-HT, histamine or bradykinin nor did it inhibit the release of inflammatory mediators from mast cells by compound 48/80. It followed, therefore, that the treatment with ellagic acid had not itself caused degranulation of mast cells. The results in Table 3 show that methysergide at the dose able to suppress carrageenin oedema did not antagonize the increase in vascular permeability caused by bradykinin or histamine but completely suppressed that due to 5-HT. Further experiments with 5-HT established that the antagonist activity of methysergide had begun to decrease after 3 h and consequently 2 h maintenance doses of methysergide were given in all experiments reported in this study.

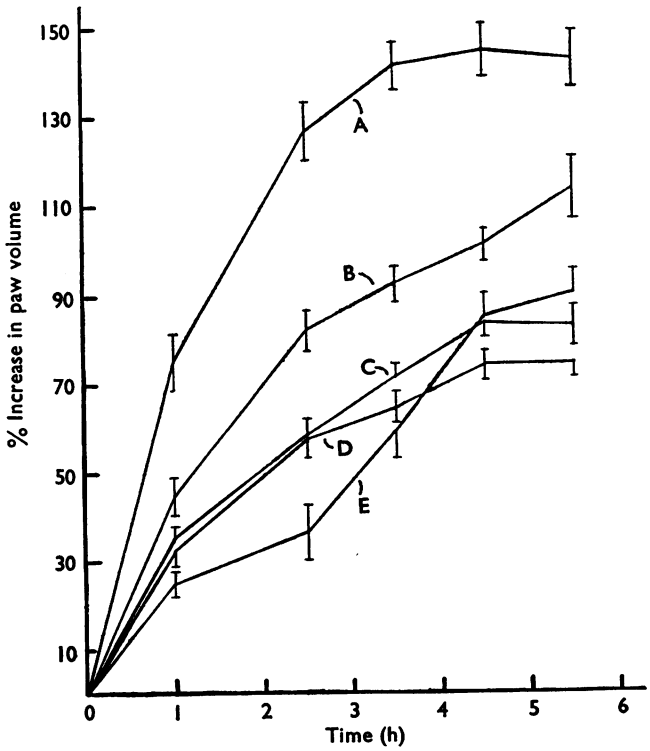


FIG. 2. Oedema formation induced by carrageenin in rats: A, untreated (twenty); B, treated with ellagic acid (nineteen); C, with methysergide (nineteen); D, with saliva (sixteen); E, with ellagic acid and saliva (twelve). Vertical bars show ±S.E.

Becker, Mota & Wong (1968) have reported that mepyramine given intravenously at a concentration of either 5 or 0·5 mg/kg partially suppresses the cutaneous permeability changes induced by bradykinin. The results in Table 4 show that we found this effect to be marginal or absent, and it was thus unlikely that mepyramine had antagonized kinins released in the carrageenin reaction. Further experiments established that a single intravenous injection of 2·5 mg/kg mepyramine suppressed the inflammatory activity of histamine for at least 6 hours.

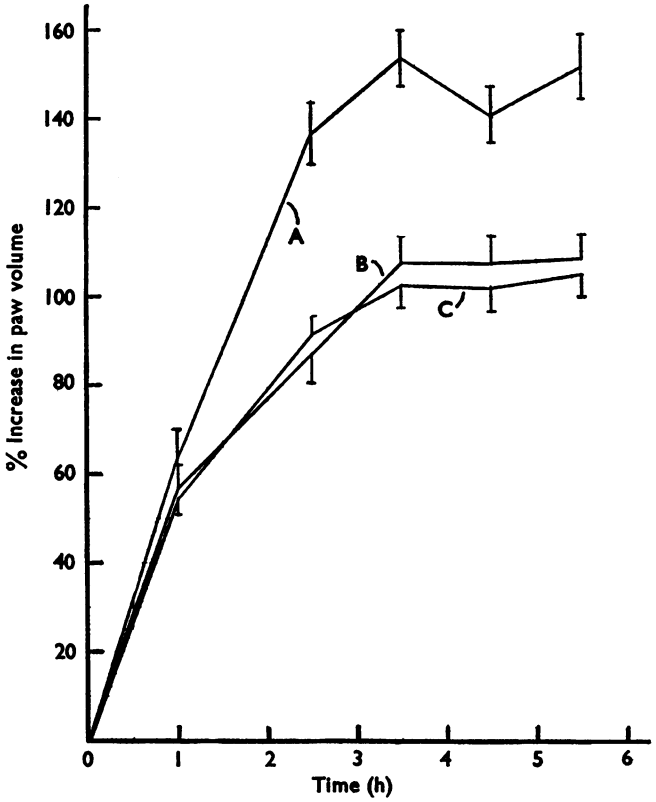


FIG. 3. Oedema formation (±S.E.) induced by carrageenin in adrenalectomized rats: A, untreated (fourteen); B, treated with ellagic acid (twenty); C, with methysergide (twenty).

TABLE 1. *Effect of ellagic acid (A), mepyramine (B) and methysergide (C), alone and in combination, on the inflammatory response of carrageenin in paws of anaesthetized rats*

| Treatment | No. of rats | Average % increase in volume (±S.E.) at 1·0 h | 2·5 h | 3·5 h | 4·5 h | 5·5 h |
|---|---|---|---|---|---|---|
| — | 29 | 93·9±5·4 | 105·6±4·9 | 112·8±5·0 | 118·8±4·4 | 120·7±4·5 |
| A | 26 | 55·5±5·3 | 53·9±4·2 | 64·9±4·3 | 76·3±4·5 | 80·9±3·8 |
| B | 19 | 93·8±5·2 | 107·2±5·5 | 110·2±5·0 | 111·0±4·6 | 108·1±5·2 |
| C | 19 | 50·2±3·3 | 62·5±3·6 | 74·5±3·5 | 69·6±2·9 | 70·4±3·9 |
| A+B | 20 | 47·3±4·2 | 42·4±3·1 | 51·5±3·7 | 63·6±4·3 | 75·1±4·0 |
| A+C | 15 | 44·8±4·3 | 49·1±5·8 | 52·1±6·4 | 60·0±6·0 | 54·5±5·6 |
| B+C | 20 | 56·1±3·2 | 62·7±2·9 | 74·2±4·0 | 74·7±5·7 | 73·6±4·1 |
| A+B+C | 16 | 33·7±3·0 | 46·0±4·2 | 48·9±3·5 | 51·9±4·2 | 54·2±5·2 |

TABLE 2. *Effect of ellagic acid treatment on the increase in vascular permeability caused by histamine, 5-HT, compound 48/80 and bradykinin in rat skin*

| Inflammatory agent (μg free base) | | Mean diameter of lesions (mm±S.E.) and dye intensity* | |
|---|---|---|---|
| | | Without treatment | After treatment |
| 5–HT | 0·2 | 16·0±1·3 (9) | 17·6±0·7 (8) |
| | 0·1 | 14·0±0·5 (8) | 14·8±0·2 (6) |
| | 0·05 | 12·8±0·5 (7) | 13·6±0·6 (6) |
| | 0·025 | 11·0±0·5 (6) | 9·8±0·7 (5) |
| Histamine | 2·0 | 13·4±0·5 (8) | 14·4±0·7 (8) |
| | 1·0 | 11·4±0·5 (7) | 11·4±0·3 (7) |
| | 0·5 | 9·2±0·3 (6) | 9·2±0·6 (6) |
| | 0·25 | 8·2±0·3 (4) | 8·0±0·3 (4) |
| Compound 48/80 | 0·1 | 16·0±0·3 (8) | 16·0±0·8 (8) |
| | 0·025 | 14·5±0·2 (6) | 13·0±1·0 (6) |
| | 0·006 | 13·2±0·3 (5) | 10·8±1·0 (5) |
| Bradykinin | 2·0 | 14·6±0·2 (8) | 15·0±0·0 (8) |
| | 1·0 | 13·6±0·2 (6) | 13·2±0·3 (6) |
| | 0·5 | 12·0±0·5 (4) | 12·8±0·5 (5) |
| | 0·25 | 10·8±0·3 (4) | 11·2±0·3 (4) |

* Intensity (in parentheses) is expressed in arbitrary units on a 0–10 scale.

TABLE 3. *Effect of methysergide bimaleate on the increase in vascular permeability caused by bradykinin, histamine and 5-HT in rat skin*

| Inflammatory agent (μg) | | Mean diameter of lesions (mm±S.E.) and dye intensity* | | |
|---|---|---|---|---|
| | | Without treatment | After methysergide (2·5 mg/kg) | After methysergide (5·0 mg/kg) |
| Bradykinin | 2·0 | 15·0±0·3 (8) | 13·8±0·3 (8) | 14·2±0·5 (8) |
| | 1·0 | 12·6±0·3 (7) | 13·3±0·5 (7) | 12·2±0·7 (7) |
| | 0·5 | 11·8±0·2 (6) | 12·3±0·3 (6) | 11·4±0·5 (7) |
| | 0·25 | 10·8±0·3 (5) | 10·5±0·3 (6) | 10·2±0·3 (5) |
| Histamine | 2·0 | 13·0±0·3 (8) | 12·2±0·7 (8) | 12·4±0·8 (7) |
| | 1·0 | 11·6±0·3 (7) | 11·6±0·5 (6) | 10·8±0·5 (7) |
| | 0·5 | 10·2±0·5 (6) | 10·8±0·8 (5) | 7·4±0·2 (5) |
| | 0·25 | 6·8±0·5 (4) | 7·8±0·2 (3) | 6·4±0·6 (4) |
| 5-HT | 0·2 | 18·4±0·2 (8) | 0·0 (0) | 0·0 (0) |

* Intensity (in parentheses) is expressed in arbitrary units on a 0–10 scale.

TABLE 4. *Effect of mepyramine maleate (2·5 mg/kg; i.v.) on the increase in vascular permeability caused by bradykinin and histamine in rat skin*

| Inflammatory agent (μg) | | Mean diameter of lesions (mm±S.E.) and dye intensity* | |
|---|---|---|---|
| | | Without treatment | After mepyramine |
| Bradykinin | 2·0 | 14·6±0·2 (8) | 13·8±0·3 (8) |
| | 1·0 | 13·6±0·2 (6) | 12·5±0·3 (6) |
| | 0·5 | 12·0±0·5 (4) | 11·5±0·3 (5) |
| | 0·25 | 10·8±0·3 (4) | 9·8±0·5 (4) |
| Histamine | 2·0 | 13·0±0·3 (8) | 0·0 (0) |

* Intensity (in parentheses) is expressed in arbitrary units on a 0–10 scale.

### *Kininogen concentrations*

Kininogen concentrations of blood were measured either 0·5 h or 2·5 h after the final injection of ellagic acid and/or rat saliva. From the results given in Table 5 it was concluded that (a) both ellagic acid (3 × 0·5 ml, 0·2 mM) and saliva (3 × 0·5 ml) caused a partial depletion of kininogen concentrations, (b) there was no significant difference between the effects of either treatment, (c) the effects of each were not additive and did not lead to a total depletion of blood kininogen and (d) kininogen concentrations had not begun to recover 2·5 h later. Injection of twice the quantity of ellagic acid into two animals had no additional effect on kininogen concentrations 30 min later.

### *Complement concentrations*

The effects on total haemolytic complement concentrations of intravenous injections of either ellagic acid or saliva, or of subcutaneous injections into the rat paw of 1% carrageenin (0·1 ml) are illustrated in Fig. 4. Ellagic acid treatment caused a temporary increase in complement concentrations which was significant at 30 minutes. On the other hand, saliva caused a 20% decrease in complement concentrations which had only recovered by 5% at 2·5 hours. Local activation of complement in the rat paw by carrageenin, if it occurred, did not lead to a decrease in serum complement concentrations. In fact a temporary increase was observed

TABLE 5. *Residual kininogen concentrations of rat plasma after injection of ellagic acid and/or rat saliva*

| Treatment | Time of sampling (h) | No. of rats | Plasma kininogen levels (Bk units/ml ± S.E.) |
|---|---|---|---|
| No treatment | 0 | 10 | 3·51 ± 0·23 |
| Ellagic acid | 0·5 | 5 | 1·05 ± 0·05 |
| Ellagic acid | 2·5 | 5 | 1·20 ± 0·08 |
| Saliva | 0·5 | 7 | 1·35 ± 0·12 |
| Saliva | 2·5 | 7 | 1·37 ± 0·24 |
| Ellagic acid, saliva | 0·5 | 7 | 1·23 ± 0·27 |
| Ellagic acid, saliva | 2·5 | 4 | 1·13 ± 0·35 |

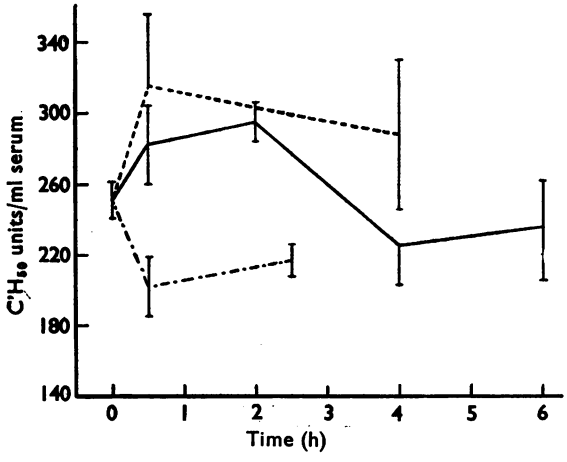


FIG. 4. Concentrations of serum complement after subcutaneous injection into the paw of carrageenin (——), intravenous injection of ellagic acid (– – –) and intravenous injection of rat saliva (–·–·–).

which was significant at 2 h ; values at 4 h and 6 h were not significantly different from concentrations before carrageenin injection.

## Discussion

Gautvik & Rugstad (1967) found that intravenous injection of ellagic acid in rats led to the elimination of kininogen substrate 1 (Jacobsen, 1966) from the circulation for at least 6 hours. They reported that blood pressure returned to normal within 10 min and that ellagic acid itself could no longer be detected in the circulation after 15 minutes. Treatment with ellagic acid was therefore expected to provide a period of almost 6 h in which to assess the contribution of kinins to the inflammatory reaction caused by an injection of carrageenin. Furthermore, this period of kininogen depletion covered the duration of the tests most widely used to assess the activity of potential anti-inflammatory drugs.

Oedema formation in rats depleted of kininogen by ellagic acid, and also by rat saliva, was considerably less than that found in untreated rats (Fig. 2). These results support the conclusions of Di Rosa & Sorrentino (1968 ; 1970) that kinins released during the inflammatory reaction had an important role in oedema formation. The larger effect of saliva was at first attributed to glandular kallikrein which would attack kininogen substrate 2, since Gautvik & Rugstad (1967) state that this substrate is not affected by ellagic acid treatment. However, measurement of plasma kininogen concentrations after treatment *in vivo* with ellagic acid or saliva (Table 5) showed that either of these reagents depleted the kininogen by an amount equivalent to more than 10 times the quantity of substrate 1 (200 ng bradykinin eq./ml plasma) measured by Gautvik and Rugstad. These results clearly indicate that treatment *in vivo* with ellagic acid causes a loss of more than substrate 1. Probably the concentrations of substrate 2 were also diminished since the effects of ellagic acid and saliva were not additive, a conclusion which is contrary to that of Gautvik and Rugstad. It is interesting that after all of these treatments a considerable amount of kininogen remained, which was presumably neither substrate 1 nor 2.

In other studies (Marsden, Crunkhorn & Meacock, unpublished results) it has been shown that maleopimaric acid, an inhibitor of complement activation (Glovsky, Becker & Halbrook, 1968), can suppress the carrageenin reaction. Carrageenin activates the complement system (Davies, 1965 ; Borsos, Rapp & Crisler, 1965 ; Willoughby, Coote & Turk, 1969) and therefore a reduction of complement concentrations by ellagic acid or saliva, if it occurred, might be expected to have an anti-inflammatory effect. Measurements of serum concentrations of complement (Fig. 4) did not indicate complement activation by the subcutaneous injection of carrageenin, but this cannot be taken as evidence that complement activation did not occur locally at the site of inflammation. Ellagic acid appeared to cause a temporary increase in complement concentrations and therefore its anti-inflammatory activity could not be attributed to these effects. Rat saliva lowered complement concentrations by approximately 20% and this difference in behaviour of ellagic acid and saliva may have contributed to their differing anti-inflammatory activity. It still remains to be explained how treatment with both ellagic acid and saliva was more effective in the first 3 h than treatment with saliva alone. The animals remained equally alert and apparently normal after all of the treatments, but it is

possible that due to effects other than those on the kinin system, the combined substances produced a greater cardiovascular response than either given singly.

Methysergide had marked anti-inflammatory activity (Fig. 2 and Table 1), from which it was concluded that 5-HT contributed substantially to oedema formation in the first few hours. These findings are contrary to those reported by Winter (1965) who used cyproheptadine and methdilazine, both of which antagonize 5-HT, but are in agreement with the results of Fekete & Kürti (1970) who used methysergide, and the results of Bhalla & Tangri (1970) who used bromolysergic acid. Experiments in adrenalectomized animals (Fig. 3) showed that the anti-inflammatory activity of ellagic acid and methysergide cannot be attributed to adrenal stimulation.

Mepyramine alone had no effect on oedema formation (Table 1), but in combination with ellagic acid treatment, with or without methysergide, it revealed that histamine played a minor role in oedema formation during the first 3 hours.

It was important to establish the degree of specific activity of the various treatments given to the rats before the induction of carrageenin inflammation. Vascular permeability studies indicated that ellagic acid treatment did not interfere with the normal responses in skin of histamine, 5-HT, bradykinin or compound 48/80 (Table 2). Also, mepyramine and methysergide, at the doses used in the carrageenin experiments, completely antagonized histamine and 5-HT, respectively, and did not affect the skin responses of bradykinin (Tables 3 and 4).

After allowing for the contributions of kinins, 5-HT, histamine and activated complement there remains a considerable inflammatory reaction not accounted for, especially after the first 4 hours. The direct effects of the mediators studied are likely to be less important after the first 3–4 h, when leucocytes emigrate into the tissue (D. A. Willoughby, personal communication). It is possible that these cells release proteolytic enzymes and also initiate the local generation of prostaglandins (Willis, 1969 ; Anderson, Brocklehurst & Willis, 1971). They might thus maintain the increased vascular permeability and could cause damage to connective tissue with the release or formation of additional unknown mediators.

We wish to acknowledge the helpful advice and criticism of Dr. W. E. Brocklehurst. Compound 48/80 was generously supplied by the Wellcome Research Laboratories, Beckenham.

## REFERENCES

ANDERSON, A. J., BROCKLEHURST, W. E. & WILLIS, A. L. (1971). Evidence for the role of lysosomes in the formation of prostaglandins during carrageenin-induced inflammation in the rat. *Pharmac. Res. Comm.*, in the Press.

BECKER, E. L., MOTA, I. & WONG, D. (1968). Inhibition by antihistamines of the vascular permeability increase induced by bradykinin. *Br. J. Pharmac.*, **34**, 330–336.

BHALLA, T. N. & TANGRI, K. K. (1970). The time course of the carrageenan-induced oedema of the paw of the rat. *J. Pharm. Pharmac.*, **22**, 721.

BORSOS, T., RAPP, H. J. & CRISLER, C. (1965). The interaction between carrageenan and the first component of complement. *J. Immunol.*, **94**, 662–666.

BROCKLEHURST, W. E. & ZEITLIN, I. J. (1967). Determination of plasma kinin and kininogen levels in man. *J. Physiol., Lond.*, **191**, 417–426.

DAVIES, G. E. (1965). Inhibition of complement by carrageenin. Mode of action, effect on allergic reactions and on complement of various species. *Immunology*, **8**, 291–303.

DI ROSA, M. & SORRENTINO, L. (1968). The mechanism of the inflammatory effect of carrageenin. *Eur. J. Pharmac.*, **4**, 340–342.

DI ROSA, M. & SORRENTINO, L. (1970). Some pharmacodynamic properties of carrageenin in the rat. *Br. J. Pharmac.*, **38**, 214–220.

FEKETE, M. & KÜRTI, A. M. (1970). Effect of monoamine oxidase inhibitors on rat paw oedema induced by various phlogistic agents. *Eur. J. Pharmac.*, **10**, 268–276.

GLOVSKY, M. M., BECKER, E. L. & HALBROOK, N. J. (1968). Inhibition of guinea pig complement by maleopimaric acid and other derivatives of levopimaric acid. *J. Immunol.*, **100**, 979–990.

GAUTVIK, K. M. & RUGSTAD, H. E. (1967). Kinin formation and kininogen depletion in rats after intravenous injection of ellagic acid. *Br. J. Pharmac. Chemother.*, **31**, 390–400.

JACOBSEN, S. (1966). Substrates for plasma kinin-forming enzymes in rat and guinea pig plasma. *Br. J. Pharmac. Chemother.*, **28**, 64–72.

ROSENBERG, L. T. & TACHIBANA, D. K. (1962). Activity of mouse complement. *J. Immunol.*, **89**, 861–867.

WILLIS, A. L. (1969). Parallel assay of prostaglandin-like activity in rat inflammatory exudate by means of cascade superfusion. *J. Pharm. Pharmac.*, **21**, 126–128.

WILLOUGHBY, D. A., COOTE, E. & TURK, J. L. (1969). Complement in acute inflammation. *J. Path.* **97**, 295–305.

WINTER, C. A., RISLEY, E. A. & NUSS, G. W. (1962). Carrageenin-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. exp. Biol. Med.*, **111**, 544–547.

WINTER, C. A., RISLEY, E. A. & NUSS, G. W. (1963). Anti-inflammatory and antipyretic activities of indomethacin, 1(*p*-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid. *J. Pharmac.*, **141**, 369–376.

WINTER, C. A. (1965). Anti-inflammatory testing methods: comparative evaluation of indomethacin and other agents. In: *Non-steroidal Anti-inflammatory Drugs*, ed. Garattini, S. & Dukes, M. N. G., pp. 190–202. Amsterdam: Excepta Medica Foundation.

(*Received March* 10, 1971)