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REVIEW

Biological properties of carrageenan

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During the past decade carrageenan has become much used experimentally mainly for its ability to induce an acute inflammation. Despite the lack of knowledge on the pathogenesis of this reaction, the fate of hundreds of new compounds as anti-inflammatory drugs still depends on their ability to suppress carrageenan foot oedema in the rat.

Nevertheless the range of the biological properties displayed by carrageenan extends much further. Thus the peculiar features of granuloma induced by carrageenan, its actions on blood coagulation and the kinin system, the contrasting observations reported on its effects on peptic ulcer, its recently discovered immunological properties as well as its interference in immune phenomena, all indicate an increasing interest in carrageenan.

Since the comprehensive review of Anderson (1967), many stimulating results in subsequent years have prompted this review with the aim of clarifying its biological properties and examining its future use as a tool to investigate physiological and pathological processes.

Occurrence

The alga *Chondrus crispus* is the main source of carrageenan. This alga is also known as carrageenin, "Irish moss" or "carragheen moss" from Carragheen (Waterford, Ireland) where it grows abundantly. The name carrageenin to designate the extract from *Chondrus crispus* was first used by Stanford in 1862 but a polysaccharide with similar properties had been isolated and described by Schmidt in 1844. The term carrageenan is more recent and has been used by several authors after 1950.

Though *Chondrus crispus* is the main source of carrageenan, material with similar composition and physical properties has been isolated from other seaweeds: *Gigartina stellata* (Dewar & Percival, 1947), *Hypnea musciformis*, *Rhodymenia palmata*, *Gracilaria confervoides* (Smith & Cook, 1953), *Eucheuma spinosum* (Houck, Morris & Lazaro, 1957; Houck, Bhayana & Lee, 1960; Anderson & Duncan, 1965; Watt & Marcus, 1970), *Furcellaria festigiata*, *Gigartina acicularis* (Houck, Morris & Lazaro, 1957; Houck, Bhayana & Lee, 1960), *Gigartina pistillata*, *Gigartina radula* (Houck, Morris & Lazaro, 1957; Houck, Bhayana & Lee, 1960; McCandless, 1962), *Polydes Rotundus* (Anderson & Duncan, 1965), *Gigartina leptorhyncus* (Johnston & McCandless, 1968).

However Tseng (1945) proposed that the name carrageenan should be restricted to the polysaccharide extracted from *Chondrus crispus* and *Gigartina stellata*.

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Chemistry

Carrageenan is a sulphated polysaccharide which has been fractionated with potassium chloride into two separate components (Smith & Cook, 1953). One fraction, which gels under the action of potassium ion, was designated κ-carrageenan, the other one, which is insensitive to potassium ion, was named λ-carrageenan. Kappa and lambda carrageenan represent respectively about 40 and 60% of the unfractionated extract.

κ-Carrageenan is composed of sulphated D-galactose and 3,6-anhydro-D-galactose residues in approximate equimolar amounts (O'Neill, 1955a), possesses a branched structure (O'Neill, 1955b) with a molecular weight between 1·8 and $3{\cdot}2 \times 10^5$ (Smith, Cook & Neal, 1954). A molecular weight of $2{\cdot}8 \times 10^5$ for κ-carrageenan was estimated by Johnston & McCandless (1968) using immunological methods.

λ-Carrageenan is composed almost entirely of sulphated D-galactose (Smith, O'Neill & Perlin, 1955) with a molecular weight between 4 and 7×10^5 (Smith & others, 1954). The molecular weight calculated by Johnston & McCandless (1968) for carrageenan was $3{\cdot}5 \times 10^5$.

Degraded carrageenan is obtained by breaking down the polymer molecules—acetolysis is the procedure usually employed which involves a partial degradation (Morgan & O'Neill, 1959). Degraded carrageenan has a molecular weight less than 30 000 and exhibits a greater stability and faster rate of dissolution than native carrageenan (Anderson, 1967).

Structure activity relations

Many of the effects displayed by carrageenan can be attributed to the polysaccharide structure and are exhibited in varying degrees by several other related compounds.

Differences between the two principle carrageenan fractions, in relation to several effects, have been often reported and the general impression is that the λ fraction seems more effective than the κ fraction. The λ fraction is more active as an irritant in eliciting either acute (Atkinson, Jenkins & others, 1962) or chronic (McCandless, 1965) inflammatory responses, as an anticoagulant (Hawkins & Leonard, 1962; 1963); it also seems more toxic (Anderson & Duncan, 1965).

McCandless (1965) examined the ability of several polysaccharides to induce granuloma formation and found that the peculiar property of carrageenan to induce inflammatory reactions was related to the chemical structural requirements that: (i) galactose must be the principal hexose with $1 \rightarrow 3$ or alternating $1 \rightarrow 3$ and $1 \rightarrow 4$; (ii) C_6 must be unsubstituted; (iii) the configuration of the galactose units plays no apparent role.

Absorption and fate

The extent of absorption of carrageenan administered by oral route remains uncertain although this polysaccharide is extensively used as a food additive in a wide range of products including cheeses, cream, chocolate and ices. Its chief use is as a suspending agent, and stabilizer, by adding body and bulk to the end product and controlling crystal formation in frozen confections. The importance of carrageenan to the food industry perhaps explains the key to the increase in interest in its absorption. Although many experimental data have been presented, in recent years, no specific

study of the absorption rate of carrageenan administered to different species has been made.

Carrageenan does not appear to be absorbed from the gut of the dog since a quantitative recovery from the stools has been reported (Houck, Bhayana & Lee, 1960). Further evidence that it is not appreciably absorbed when ingested has been provided by young rats fed with native or degraded carrageenan (Dewar & Maddy, 1970). However when native or degraded carrageenan (from *Eucheuma spinosum*), labelled with ferric iron, was added to the diet of guinea-pigs for 1 week, it was possible to demonstrate particulate Perls' positive granules within the macrophages and epithelial cells found in the subepithelial layers of the caecum; no labelled material was found in the ileum or colon (Sharratt, Grasso & others, 1970). In the guinea-pig, carrageenan may be absorbed after intraperitoneal injection because it has a suppressive effect on delayed hypersensitivity when given by this route (Schwartz & Leskowitz, 1969). However these authors were unable to distinguish between the non-specific effect of the intraperitoneal treatment and a true immunosuppressive activity. Davies (1963) was unable to detect any fall in total haemolytic complement titre of the guinea-pig after intraperitoneal injection of an amount of carrageenan four times larger than the dose required to produce a fall in C′ titre when given intravenously.

Subcutaneously, carrageenan has a very poor absorption rate in rat and guinea-pig and this may be related to its inflammatory effects. Thus Spector & Ryan (1969) have found carrageenan to persist in long-lived macrophages for periods of months after injection. For the rat, however, a single massive subcutaneous dose of carrageenan may be partially absorbed, as this treatment elicits hypersensitivity to cold and haemorrhagic necrosis of transplanted lymphosarcomas (Selye, 1965). Degraded caraageenan seems to be absorbed subcutaneously in the rat because it renders some protective effect towards ulcerogenesis according to Eagleton, Watt & Marcus (1969). Of its fate in the organism all that is known is that particles of polysaccharide are actively phagocytosed by monocytes and macrophages and can be detected in these cells for long periods (Williams, 1957; Allison, Harrington & Birbeck, 1966).

Clear cut conclusions are thus not easily reached especially as there is often a lack of information on the source of carrageenan and the dose employed and the lack of detailed knowledge about any particular species.

Toxicity

Investigations of the toxicity of carrageenan like those of absorption often leave much to be desired. There is a paucity of information on the source of carrageenan and often the data presented represent incidental findings by various investigators rather than a complete toxicological study.

By the oral route carrageenan (5 mg/ml in drinking water) seems well tolerated in dogs after 30 days of chronic dosage (Houck & others, 1960). In rabbits degraded carrageenan added to drinking water (0·1–1·5%) over a 6–12 week period caused diarrhoea, severe ulcerations of colon and loss of weight according to concentration (Watt & Marcus, 1970). Similar observations had previously been reported in guinea-pigs (Watt & Marcus, 1969). Lethal effects of carrageenan had been observed only after intravenous injections. Carrageenan from *Gigartina acicularis* (15 mg/kg) caused the death of 2 out of 3 dogs within 24 h (Houck, Morris & Lazaro, 1957). In a study on anticoagulant activity of carrageenan in rabbits, Anderson & Duncan (1965) found the lowest intravenous single doses to produce death within 24 h to be

1–5 mg/kg for λ-carrageenan and 3–15 mg/kg for κ-carrageenan, both isolated from *Chondrus crispus*. In contrast, repeated intravenous doses of approximately 15 mg/kg either of λ-carrageenan *(Chondrus crispus)* or of κ-carrageenan to rabbits *(Chondrus crispus* and *Cigartina leptorhyncus)* were well tolerated (Johnston & McCandless, 1968), while no deaths occurred after intraperitoneal injection of about 300 mg/kg carrageenan in the guinea-pig (Schwartz & Leskowitz, 1969) or after subcutaneous infiltration of about 2000 mg/kg in the rat (Selye, 1965).

INFLAMMATORY ACTIVITY

Oedema

The use of carrageenan as an irritant to induce oedema formation in the rat foot was first introduced by Winter, Risley & Nuss (1962). Foot swelling was elicited by subplantar injection of 0·05 ml of 1% carrageenan suspension in saline. Foot volume was measured immediately and 3 h after the injection. The difference was recorded as "volume of oedema". The anti-inflammatory effect of indomethacin was assayed using this procedure (Winter, Risley & Nuss, 1963) which with slight modifications has become popular as a test for anti-inflammatory activity.

The value of this model of acute inflammation as a test system for new anti-inflammatory drugs received support from a report by Niemegeers, Verbruggen & Janssen (1964) who after analysing the inhibitory effect of a number of drugs with or without established clinical antirheumatic effect, concluded that the assay was an acceptable preliminary screening test for antirheumatic activity.

Another advantage of carrageenan oedema in comparison with the oedema elicited by other phlogistic agents, was its responsiveness to doses well below the toxic level (Winter & others, 1963). It is interesting that as virtually all the assessments were based on the 3 h oedema, carrageenan oedema was regarded as a test unique in its susceptibility to antirheumatic drugs and some confusion arose about its pathogenesis and the role of the mediators involved. As the acute inflammatory response is brought about by several mediators released in ordinate sequence (Spector & Willoughby 1963, 1965; Willoughby & Di Rosa, 1971) it seemed difficult to obtain a satisfactory explanation of the whole reaction by limiting the observations to a discrete time. It also seemed illogical that a model of acute inflammation should be effective as a test system for assessing the activity of drugs in chronic inflammation, especially as the acute system measures oedema and the chronic process involves cellular emigration to a greater extent. The time course of carrageenan oedema has been described by Van Arman, Begany & others (1965) but only recently Vinegar, Schreiber & Hugo (1969) and Di Rosa, Giroud & Willoughby (1971) emphasized the presence of different phases in the development of the oedema, although with different conclusions about the role played by the mediators in each one.

The role of histamine or 5-hydroxytryptamine (5-HT) was denied by several investigators (Winter & others, 1963; Van Arman & others, 1965; Vinegar & others, 1969). All these authors based their conclusions on the failure of antihistamines or of 5-HT antagonists, given singly, to modify the oedema. However, by depleting rats of their histamine and 5-HT stores with compound 48/80, Di Rosa, Giroud & Willoughby (1971) were able to suppress the oedema for the first 90 min.

Similarly, a combined treatment of the animals with an antihistamine and a 5-HT antagonist resulted in a marked suppression of this early phase of inflammation.

This simultaneous release of two mediators explains the failure of other authors to implicate histamine or 5-HT in this reaction. Vinegar & others (1969) ascribe this first phase to bradykinin release, supporting this concept with the findings that those anti-inflammatory drugs that inhibit the first phase are also capable of antagonizing bradykinin induced lung constriction (Collier & Shorley, 1960). We would only observe that from the data presented by these authors the same drugs appear more effective in inhibiting the second phase which according to Di Rosa & others (1971) is brought about by kinin release.

The implication of the kinin system in the pathogenesis of carrageenan oedema was hypothesized on the basis that oedema is suppressed by antiproteasic agents, either injected locally (Van Arman & others, 1965) or systemically administered (Di Rosa & Sorrentino, 1968) as well as on the ability of carrageenan to release kinins when it is incubated *in vitro* with plasma substrates (Rothschild & Gascon, 1966; Di Rosa & Sorrentino, 1968). Further evidence in support of this concept was the recent finding by Di Rosa & Sorrentino (1970) that treatments of rats with cellulose sulphate, a kininogen-depleting agent, led to a suppression of the oedema which was closely related to the time course of kininogen depletion. Recently it was established that kinins bring about the reaction for a discrete period, i.e. between $1\frac{1}{2}$ and $2\frac{1}{2}$ h (Di Rosa & others, 1971). During this intermediate phase the foot swelling was enhanced by treating rats with kininase inhibitor 1,10-phenanthroline (Sorrentino & Capasso, 1971). The transient role of kinins, related to this 2nd phase may be the reason why some confusion has arisen over the role of kinins. Thus, Van Arman has in turn supported the role of kinins as mediators of the reaction (Van Arman & others, 1965) and more recently refuted their importance (Van Arman & Nuss, 1969).

A third phase ($2\frac{1}{2}$–6 h) in the development of carrageenan oedema was recently described by Di Rosa & others (1971). This phase is brought about by mediators other than histamine, 5-HT and kinins, and this is thought to be associated with prostaglandins or a mixture of PG and SRS (Spector & Willoughby, 1962). The implication of many mediators in the carrageenan oedema was in agreement with the findings of Willis (1969a, b) who by using a cascade superfusion technique was able to detect the release of histamine, 5-HT, kinins and prostaglandins in carrageenan induced exudates.

From recent investigations (Dias da Silva & Lepow, 1967; Willoughby, Coote & Turk, 1969) it has been put forward that complement system should act as a trigger mechanism of non-immune inflammation.

In complement-depleted rats, oedema was depressed for a 6 h period following injection of carrageenan (Di Rosa & others, 1971), it seems conceivable that the whole inflammatory response promoted by carrageenan is brought about by local activation of the complement system. This assumption is also supported by experiments in which the kininogen depletion caused by cellulose sulphate was dissociated from the anti-complementary activity exhibited by this compound (Di Rosa & others, 1971). When cellulose sulphate was administered 3 h before carrageenan, so that the complement system was completely restored, while kininogen was still depressed, the inhibition of the "kinin phase" was very evident compared with the more non-specific inhibition observed when cellulose sulphate was administered shortly before carrageenan (Di Rosa & Sorrentino 1970). Then the foot swelling that occurs after $2\frac{1}{2}$ h was masked by the simultaneous lowering of the complement system caused by cellulose sulphate, which led to a longer lasting suppression.

The implication of complement in carrageenan inflammation should also explain the inhibition of the oedema after intravenous injection of heparin or plasmin (Wiseman & Chang, 1968). It is known in fact that heparin exhibits anti-complement activity (Ecker & Pillemer, 1941) as well as that plasmin is able to inactivate human complement (Ratnoff & Naff, 1967).

Thus the third phase is almost neglected in the 3 h assessment of carrageenan oedema. Yet this phase 2½–6 h appears to be the most interesting compared with the two earlier phases. Thus, the maximal vascular response as determined with the accumulation of ^{131}I albumin into the inflamed foot, occurs during this phase (Di Rosa & others, 1971); cell migration, mainly of polymorphonuclear cells, also reaches its maximum level in this third phase (Di Rosa, Papadimitriou & Willoughby, 1971).

From this investigation on the time course of the cellular exudate in carrageenan foot odema, it has been found that the cellular migration virtually starts at the 2nd h, when few cells (10–20 cells per high power random field), mainly surrounding the vessels, are detectable.

At 4 h a well-defined and highly developed cellular exudate (about 100 cells per high power random field) was observed consisting mostly of polymorphonuclear cells (about 70%) and mononuclear cells (about 30%) with the occasional presence of fibroblasts and macrophages. At 6 h the cellular exudate exhibited the same character but the number of cells was still further increased (about 150 cells per high power random field) without significant modification in the ratio of polymorphonuclear and mononuclear cells.

As the later phase of carrageenan oedema (2½–6 h) displays the two main features of the inflammatory process, namely vascular response and cellular emigration, the most important non-steroidal antirheumatic drugs have been tested on this phase (Di Rosa & others, 1971).

Aspirin, butazolidin, indomethacin and mefanamic acid, administered orally to rats previously depleted of their histamine, 5-HT and kininogen stores, resulted in a large depression of the oedema that develops in these rats between 3 and 6 h. The depression of foot swelling was constantly accompanied by inhibition of the emigration of cells (mainly monocytes) into the inflamed area.

These results, which stress the close connection between the later phase mediators (suspected to be prostaglandins or SRS) and cell emigration, have support in the recent findings of Vane and his colleagues on the action of prostaglandins and should lead to a better understanding of the mechanism of action of non-steroidal anti-inflammatory drugs as well as to a more rational approach to their assessment. This concept is supported by further data showing that both dextran and formalin, two irritants that have been virtually replaced by carrageenan, when injected into the foot of rats depleted of their stores of histamine, 5-HT and kininogen, fail to elicit either a good increase of vascular permeability or leucocyte migration (Di Rosa & Willoughby, 1971).

Pleurisy

Carrageenan was also used in the rat as an irritant to induce inflammatory exudates in sites other than the foot. A pleurisy induced by a mixture of Evans-blue and carrageenan was proposed by Sancilio (1969) as a model for the assay for non-steroidal anti-inflammatory drugs. Carrageenan pleurisy was also studied by Di Rosa & others (1971) who found neither substantial difference in the pattern of the mediators

in comparison with the foot oedema, nor with the response to other irritants, e.g. turpentine.

Pouch

Several authors have proposed carrageenan-induced subcutaneous abscess in the rat as a test for the assay of anti-inflammatory drugs either by subcutaneous injection (Benitz & Hall, 1963; Goldstein & Schnall, 1963) or by implantation of carrageenan tablets (Atkinson & others, 1962; Boris & Stevenson, 1964).

Granuloma

Robertson & Schwartz (1953) demonstrated that carrageenan injected subcutaneously in the guinea-pig was able to induce the development of a granulomatous tissue containing large amounts of collagen. The granulomatous tissue formation is maximal at about 1 week and is completely resorbed at 46 weeks (Jackson, 1957; Slack, 1957). The maximal concentration of collagen has been observed on 14th day, most of the insoluble collagen being formed between the 5 and 9th day. According to Lowther, Green & Chapman (1961) microsomes seem to be the site of the initial accumulation of collagen.

Carrageenan granuloma has been used by several investigators to elucidate the role of ascorbic acid (Robertson & Hinds, 1956; Robertson, Hiwett & Herman, 1959; Slack, 1958) as well as of hormonal factors (Robertson & Sanborn, 1958; Fisher & Paar, 1960) in collagenogenesis. The cellular evolution of carrageenan granuloma in the guinea-pig has been described by Williams (1957). Carrageenan stimulates a moderate polymorphonuclear response followed on the 5th day by a granulomatous reaction brought about by actively dividing fibroblasts intimately mixed with histiocytes. The new connective tissue, which matures into adult collagen on the 14th day, is afterwards broken down and completely resorbed within 6 weeks. Resorption is accompanied by a loss of collagen fibres from the overlying dermis which is replaced by adipose tissue. The mechanism of this replacement, a peculiar feature of carrageenan granuloma, remains unknown. The cellular sequence of carrageenan granuloma in rats, according to Benitz & Hall (1959) and Morris, Weinberg & Spector (1968) differs from that described for guinea-pigs, thus there is a more pronounced acute inflammatory response with larger numbers of polymorphonuclear cells emigrating into the tissues during the first 24 h. Later, the lesion is characterized by the occurrence of many histiocytes and marginating monocytes, a small number of fibroblasts also being present. An intense histiocyte proliferation and macrophage differentiation was also found and provided a simple tool for the study of structure and function of macrophages.

Carrageenan is rapidly taken up within macrophages (Allison, Harington & Birbeck, 1966) and is virtually present in all reacting cells (Ryan & Spector, 1969). The inability or the difficulty by macrophages to digest carrageenan may provoke an impairment of some of their functions and explain the peculiar feature of carrageenan granulomata in the rat which is characterized by long-lived macrophages with a low turnover rate (Spector, 1969). Chronic granulomata have been classified into two groups, "proliferative" or "high turnover" granulomata and "inert" or "low turnover" granulomata (Ryan & Spector, 1969; Spector, 1969). In both types the marrow-derived macrophages play a predominant role. A further common feature of both

these forms of chronic inflammation is the retention of irritant within the cytoplasm of macrophages.

The proliferative granuloma is characterized by presence of irritant only in a proportion of mononuclear cells and by high rates of macrophage turnover and migration. The inert granuloma is in contrast characterized by the presence of irritant in all macrophages and by low rate of macrophage turnover and migration. The lesion induced by *Bordetella pertussis* is an example of proliferative granuloma whereas the lesion caused by carrageenan provides an example of inert granuloma.

EFFECTS ON THE KININ SYSTEM

In vitro

The ability of carrageenan to release kinins when incubated with plasma substrates was reported by Rothschild & Gascon (1966) and was attributed to the activation of a proteolytic process. The kinin formation by plasma enzymes is brought about by kallikrein as well as by plasmin (Henriques, Lavras & others, 1966). Release of kinin-like substances was observed when carrageenan was incubated with plasma-heated for 3 h at 56° (Di Rosa & Sorrentino, 1968). As this treatment inactivates plasma kallikreinogen (Schachter, 1956) these results suggest that carrageenan promotes kinin formation by activating plasmin. This concept is also supported by recent findings by Schwartz & Kellermeyer (1969) which show that carrageenan activates Hageman factor; it is known that activation of Hageman factor leads in turn to an activation of plasmin system (Iatridis & Ferguson, 1965).

In vivo

Intravenous administration of carrageenan in the rat caused intense hypotension (Di Rosa & Sorrentino, 1970). The fall was of tachyphylactic type as subsequent administrations of carrageenan elicited progressively reduced effects and became completely ineffective. This effect, unaffected by histamine antagonists, and abolished by protease inhibitors, was attributed to kinin release from plasma substrates. This concept was supported by the enhancement of the esterolytic activity of the blood (Di Rosa & Sorrentino, 1970) as well as by the fall of the plasma kininogen level (Van Arman & Nuss, 1969) both observed after intravenous administration of carrageenan in the rat. However as an interference of carrageenan with complement system has been described (see p. 97) it is conceivable that several other vasoactive factors can be formed by this route. Thus the mechanism of carrageenan effects on blood circulation appears more complicated as different factors in addition to kinin release could be involved.

EFFECT ON COAGULATION SYSTEM

Anticoagulant activity of carrageenan extracted from *Gigartina acicularis* was described by Houck & others (1957) who found whole extracts prepared from several other seaweeds to be ineffective. Unfractionated carrageenan derived from *Chondrus crispus* as well as both its κ and λ fractions exhibited anticoagulant activity (Hawkins & Leonard 1962, 1963). The most effective was the λ fraction which was 1/15 as active as heparin in doubling the thrombin time in both dog and human plasma. As the thrombin time increased with increasing concentration of carrageenan, it was deduced that the anticoagulant effect was of antithrombotic origin. However,

Anderson & Duncan (1965), after analysing the anticoagulant activity of carrageenan on a number of clotting tests, suggested that it might affect the early stages of clotting process. This was supported by recent findings that it is able to activate Hageman factor in human plasma (Schwartz & Kellermeyer, 1969). As carrageenan produced a dual dose-dependent effect on clotting time (anticoagulant at higher concentrations and pro-coagulant at lower ones) it seems that the whole problem of the interference between carrageenan and the coagulant system has yet to be elucidated.

EFFECTS ON COMPLEMENT SYSTEM

Inhibition of haemolytic complement of various species both *in vitro* and *in vivo* was described by Davies (1963, 1965) and was attributed to the ability of carrageenan to prevent the fixation of C'1 on the sensitized red blood cells or to its interference with a component of complement that precedes the fixation of C'1. The same conclusion was reached by Borsos, Rapp & Crisler (1965) with guinea-pig complement although the degree of inhibition these authors observed *in vivo* was very low compared to that reported by Davies (1963). However, Ward & Cochrane (1965) found that rat and guinea-pig complement are affected by carrageenan predominantly by interference with the activity of the first two reacting components C'1, 4.

ANTI-INFLAMMATORY AND IMMUNOSUPPRESSIVE EFFECTS

Effect on acute inflammation

By treating rats with carrageenan, Willoughby & others (1969) were able to inhibit two models of acute non-immune inflammation—those of turpentine pleurisy and thermal injury. Carrageenan at the dose level employed induced a depression of the complement system which related well with the degree of inhibition of inflammatory responses. These experiments gave further support to the implication of the complement system in non-immune inflammation.

Effect on peptic ulcer

The anti-ulcerogenic activity of carrageenan has been extensively studied and has been mainly related to its antipeptic properties. However, more specific anti-inflammatory properties have been attributed to carrageenan in relation to its ability to remove that part of the kininogen system that can be activated (Rocha e Silva, Cavalcanti & Reis, 1969). Degraded carrageenan given orally to guinea-pigs depressed volume, peptic activity and acidity of gastric juice and gave a marked protection against duodenal ulcer and a mild protection against gastric ulcer, both induced by histamine, without any detectable histological change in the gastric mucosa (Anderson & Watt, 1959a, b; Anderson, Marcus & Watt, 1962). The protective properties displayed by carrageenan were attributed to a local effect related to its ability in preventing the diffusion of pepsin through the mucin (Anderson, 1961), but a more complex mechanism involving a systemic response was also suggested (Anderson & Soman, 1963).

Oral carrageenan, according to Houck & others (1960), markedly suppressed ulcerogenesis in rats with pyloric ligation or given subcutaneous injection of cortisone, and in dogs subjected to large doses of histamine. A competitive inhibition *in vitro* by carrageenan on the proteolytic activity of pepsin was also reported (Houck &

others, 1960; Bonfils, Dubrasquet & Lambling, 1960). As neither toxic side-effects after chronic dosage, nor anticoagulant effect were found, the use of carrageenan in the clinical management of ulcer was predicted.

Degraded carrageenan was administered to patients affected by peptic ulcer with some alleged successful results (Bonfils & Lambling, 1960; Berthet, 1961; Heineken, 1961; Esposito & Nicolini, 1961; Esposito, 1962). I consider these results should be regarded with caution as they were inadequately controlled. Thus a blind trial on the effectiveness of degraded carrageenan in the treatment of peptic ulcer led to the conclusion that it appeared effective in giving symptomatic relief but failed to influence the natural history of the disease (Evans, Nowell & Thomas, 1965).

However, different results were reported by other, and sometimes by the same, investigators. Thus carrageenan failed to protect dogs against cinchophen ulcer (Figueroa & Klotz, 1963) and similarly Eagleton, Watt & Marcus (1969) were unable to find any protective effect on gastric ulcer after subcutaneous administration of degraded carrageenan which displayed only a mild protection on duodenal ulcer. Other investigations showed that ulcerative lesions can be produced in guinea-pigs and rabbits after administration of degraded carrageenan (from *Eucheuma spinosum*) solubilized in drinking water over a 6–12 weeks period (Watt & Marcus, 1969, 1970). These reports by Watt & Marcus gave rise to a variety of reactions and prompted several authors to disclose their results and to present their comments in the correspondence columns of the Lancet. Bonfils (1970) denied that oral administration of carrageenan may be a hazard in man as none of the 200 cases of peptic ulcer treated with 5 g daily of degraded carrageenan (Ebimar) presented any evidence of additional gastrointestinal disease throughout the period of the treatment, which ranged from 6 months to 2 years. An investigation of the effects of carrageenan in animals of several species (guinea-pigs, rats, mice) has been made by Maillet, Bonfils & Lister (1970) who found no evidence of any pathological changes in rats and mice. In contrast, their guinea-pigs exhibited mucosal erosions in the caecum and, in rare instances, in the colon. These results agree with the findings of Sharratt & others (1970) who, using carrageenan labelled with ferric iron, were able to demonstrate that in the guinea-pigs carrageenan passes through the caecal epithelium, but not through the colon or ileum. Thus, at present, it seems conceivable that the ulcerative phenomenon is restricted to guinea-pigs, or more generally to herbivorous animals possessing a special ability to absorb carrageenan. Although differences in the source of carrageenans, in their physicochemical properties (native or degraded), in the dose and in the length of the treatment, and in the various animal species used could explain the discrepancies observed, further studies on the action of carrageenan on the gastrointestinal tract would seem to be needed.

Effects on immediate hypersensitivity

Carrageenan repeatedly administered both by intravenous and intraperitoneal routes was found to inhibit the Arthus reaction in both rats and guinea-pigs (Ward & Cochrane, 1965). This effect was attributed to the ability of carrageenan to inhibit the complement system. But considering the high dose employed (the total amount of administered polysaccharide was about 100 mg per animal) and the consequent lethargic state of the animals, a non-specific effect due to the toxicity of carrageenan should not be excluded. On the other hand intravenous administration of carra-

geenan to guinea-pigs, did not affect their susceptibility to acute or protracted anaphylactic shock (Davies, 1965).

Effects on delayed hypersensitivity

Carrageenan has been reported (Schwartz & Leskowitz, 1969) to inhibit delayed hypersensitivity when administered to tuberculous sensitive guinea-pigs. The suppression was dose-dependent and not related to anticomplementary or anticoagulant activity. This effect of carrageenan was attributed to an impairment of macrophages (Allison, Harrington & Birbeck, 1966), which became unresponsive to the factors released by the reaction between antigen and sensitized lymphocytes (Humphrey, 1967). However, the activation and the possible depletion of Hageman factor promoted by carrageenan has also been emphasized as a possible explanation and consequently a role of Hageman factor in delayed hypersensitivity has been put forward (Schwartz & Kellermeyer, 1969).

IMMUNOGENIC PROPERTIES

Relatively small amounts of both κ- and λ-carrageenan fractions brought about the formation of precipitating antibodies in rabbits (Johnston & McCandless, 1967, 1968). The kinetics of the reaction of the carrageenan-anticarrageenan system were similar to those of other polysaccharide-antipolysaccharide systems. It was interesting that both κ and λ fractions showed a high degree of immunologic specificity because no cross reaction was observed between κ-carrageenan and anti-λ serum as well between λ-carrageenan and anti-κ serum.

Sensitization of the guinea-pig to carrageenan has been described by McCandless (1967). Guinea-pigs pre-treated subcutaneously with unfractionated or with λ-carrageenan exhibited a sensitization reaction when challenged 3, 5, or 8 weeks later with the same polysaccharide. Granulomata from sensitized animals were consistently larger (about 2 fold weight), more haemorrhagic and had a significantly lower collagen concentration compared with granulomata from animals pretreated with saline. Total protein, dry defatted solid and iron concentrations were not significantly altered so that the increase in granuloma weight exhibited by sensitized animals was mainly attributed to a large increase of these elements and especially of non-collagenous protein synthesis.

Conclusions

From the above observations carrageenan emerges as a fascinating compound having a wide spectrum of interference with biological systems.

An extensive and systematic investigation of the absorption and toxic effects exhibited by carrageenan seems a real need in view of its increasing use in pharmaceutical, cosmetic and dairy industries.

Carrageenan induces acute (oedema) and chronic (granuloma) inflammatory responses. It is a unique and surprising feature that, compared to the granuloma, the acute response provides a more appropriate model for the assay of antirheumatic drugs which are directed against a chronic process. This is mainly due to the marked cellular emigration which occurs during the acute response compared with the low turnover rate of cellular proliferation and entry which is observed in the granuloma. Thus, the acute response appears the more similar to rheumatoid arthritic lesions, which are characterized by sustained cellular emigration.

The ability of non-steroidal antirheumatic drugs to suppress carrageenan oedema seems closely related to their ability to prevent cell emigration. Broadly speaking the measure of oedema inhibition is actually a measure of cell emigration inhibition.

The immunosuppressive and immunogenic properties of carrageenan are too recent and the findings too incomplete to permit proper conclusions. However it does seem reasonable to predict that carrageenan will prove a useful tool to investigate the relationship between immune and non-immune inflammation as well as the still obscure transition from acute to chronic inflammation.

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