Carrageenan and Delayed Hypersensitivity
II. Activation of Hageman Factor by Carrageenan and Its Possible Significance[1] (34358)

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Carrageenan is a high molecular weight, long-chain polymer of sulfated galactose units that can be extracted from marine plants. Carrageenan has recently been shown to suppress the delayed hypersensitivity reactions of sensitized guinea pigs (1), inhibit the complement system (2, 3), damage macrophages *in vitro* (4) and *in vivo* (5), and induce kinin formation in rat plasma (6).

In this paper we show that carrageenan activates Hageman factor (factor XII) in human plasma, and thus promotes blood coagulation, induces vascular permeability, and liberates kinin (7). At higher concentrations it also acts as an anticoagulant, as reported previously (8).

Materials and Methods. Carrageenan was kindly supplied by Marine Colloids, Inc., Springfield, New Jersey as Seakem 21. The powder was dissolved in hot saline prior to use, using a double boiler technique.

Human platelet-deficient plasma was prepared in silicone coated plastic equipment. Venous blood was drawn into 30-ml plastic syringes, mixed with 0.1 vol of 0.13 *M* sodium citrate pH 5.0 in 40-ml Lusteroid tubes and centrifuged 30 min at 700*g* at 1°. Plasma was then transferred to thick-walled silicone coated Servall centrifuge tubes and centrifuged at 30,000*g* for 30 min. Plasma aliquots were then transferred to plastic, silicone coated containers and stored in the frozen state until use.

PTA-deficient, Christmas factor-deficient, and Hageman factor-deficient plasma was kindly provided by Dr. Oscar D. Ratnoff.

Barbital–saline buffer and DeJalon solution was prepared as described elsewhere (9, 10). *Soybean trypsin inhibitor* (SBTI) (Worthington) was freshly prepared in barbital–saline buffer at desired concentrations. *O*-Phenanthroline (Fisher) was prepared in barbital–saline buffer (2.5×10^{-3} *M*) and stored at 4° for less than 2 weeks. *Hexadimethrene bromide* (Polybrene, Abbott Laboratories) was similarly prepared and stored. *Anti-Hageman factor globulin* was prepared as described previously (11) and kindly provided by Dr. Oscar D. Ratnoff.

Vascular permeability enhancing activity was assessed by calculating the mean diameter (mm) of the blue spot developed after intradermal injection of 0.1 ml of test material into each of 4 guinea pigs pretreated with Pontamine sky blue (11, 12).

Recalcified clotting time of platelet-deficient plasma was measured in 10 × 75-mm polystyrene test tubes (Falcon) in duplicate as previously described (13).

Development of kinin activity in plasma was measured by methods previously described (14), using the isolated rat uterus.

Results. I. The effect of carrageenan on blood clotting. Carrageenan had a dual, dose-dependent effect on the recalcified clotting time of normal human plasma. Anticoagulant activity was seen at concentrations greater than 10^{-3} mg/ml, whereas procoagulant activity was seen at lower concentrations. Maximum procoagulation was seen at 3.3×10^{-5} mg/ml, at which level the recalcified clotting time was reduced to approximately ½ of the control (Table I).

When plasma deficient in Hageman factor was used, prolonged clotting times were again noted at high concentrations (Table II) and

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TABLE I. The Effect of Carrageenan on the Recalcified Clotting Time of Normal Human Platelet-Deficient Plasma.

Test substance		Clotting time (min)
Buffer		28.5
Carrageenan[a] (mg/ml)	3.3	>75.0
	3.3×10^{-1}	>75.0
	3.3×10^{-2}	>75.0
	3.3×10^{-3}	34.0
	3.3×10^{-4}	22.7
	3.3×10^{-5}	12.0
	3.3×10^{-6}	17.0
	3.3×10^{-7}	20.5
	3.3×10^{-8}	22.7

[a] Final concentrations in a total volume of 0.3 ml containing 0.1 ml of plasma and 0.1 ml of $CaCl_2$.

only slight decrease in the recalcified clotting time was seen at lower concentrations. No clot-promoting activity was seen in cow plasma deficient in PTA, or in human plasma deficient in Christmas factor.

When hexadimethrene bromide (2×10^{-1} and 5×10^{-2} mg/ml) was added to normal human plasma, the procoagulant effect of carrageenan was blocked. This effect was less apparent at a concentration of 2×10^{-2} mg/ml (Table III).

II. The effect of carrageenan on permeability enhancing activity in plasma. Incubation of carrageenan (3.3×10^{-4} mg/ml) with normal human plasma led to the development of permeability enhancing activity (Table IV). This effect was prevented by the prior addition of SBTI (Table IV) to normal plasma, and was not obtained when Hageman factor-deficient plasma was used. The intradermal injection of carrageenan (3.3×10^{-1}) alone did not lead to increased vascular permeability.

III. The effect of carrageenan on kinin formation. Incubation of carrageenan (3.3×10^{-1}) with normal human plasma led to activation of the kinin system, as measured by smooth muscle contraction in the rat uterus assay system (Table V), This result was abolished by addition of SBTI to the incubation mixture.

When carrageenan was incubated with Hageman factor-deficient plasma, kinin activity did not develop. When normal human plasma was preincubated with anti-Hageman factor globulin, and carrageenan (3.3×10^{-1} mg/ml) later added, the development of kinin activity was suppressed, with only ⅓ of the control contraction attained.

Discussion. It has been shown that activation of Hageman factor in normal human plasma results in accelerated blood clotting, and in the development of both kinin and vascular permeability enhancing activity as well (7). In the present paper we confirmed previous reports that carrageenan has anticoagulant (8) and kinin-releasing activities (6). In addition, we showed that carrageenan can also act as a procoagulant and can bring about enhanced vascular permeability.

We feel that these latter three effects are the result of the activation of Hageman factor by carrageenan. They develop in normal plasma, but fail to develop in Hageman factor-deficient plasma. SBTI and hexadimethrene bromide are known to interfere with the consequences of Hageman factor activation (Ref. 7). Thus, the suppression of the procoagulant, kinin releasing, and vascular permeability enhancing activities in normal plasma of carrageenan by these materials (Tables III–V) and the inhibition of kinin activation by anti-Hageman factor globulin (Table V) are consistent with this interpretation.

Carrageenan has several other interesting biological effects as well. It inhibits the complement system, by interfering with the capacity of C'1 to become bound to sensitized

TABLE II. The Effect of Carrageenan on the Recalcified Clotting Time of Hageman Factor-Deficient Human Platelet-Deficient Plasma.

Test substance		Clotting time (min)
Buffer		69.0, 72.0
Carrageenan[a] (mg/ml)	3.3×10^{-1}	>120.0, 115.0
	3.3×10^{-3}	40.5, 51.5
	3.3×10^{-5}	31.0, 42.0
	3.3×10^{-6}	38.0, 43.0
	3.3×10^{-7}	54.0, 49.5
	3.3×10^{-8}	48.5, 46.5

[a] Final concentrations.

TABLE III. The Effect of Hexadimethrene Bromide on the Clot-Promoting Activity of Carrageenan.

Mixture	Recalcified clotting time of normal plasma[a] (min)
Buffer + normal plasma	24, 26
Carrageenan (2.5×10^{-4} mg/ml) + normal plasma	11, 11
Hexadimethrene (5×10^{-2} mg/ml) + normal plasma	85, 85
+ carrageenan (2.5×10^{-4} mg/ml) + normal plasma	85, 85
(1.25×10^{-2} mg/ml) + normal plasma	50, 52
+ carrageenan (2.5×10^{-4} mg/ml) + normal plasma	50, 52
(5×10^{-3} mg/ml) + normal plasma	31, 31
+ carrageenan (2.5×10^{-4} mg/ml) + normal plasma	23, 25

[a] In a total volume of 0.4 ml, containing 0.1 ml of each reagent plus sufficient buffer to make up to final volume. Concentrations indicated are final in volume used.

erythrocytes (3). It also is an immunogen, causing the formation of precipitating antibodies in rabbits (15), and in a growth stimulant for connective tissue (16).

Of what biological significance may these properties of carrageenan be? Davies showed no alteration in the susceptibility of carrageenan-treated animals to anaphylaxis, although a significant decrease in serum complement levels were observed (2). It has been demonstrated previously that administration of carrageenan to tuberculin-sensitive guinea pigs results in a dose-dependent suppression of delayed hypersensitivity which was not correlated with anti-complementary or anticoagulant activity (1). It was suggested that the suppressed delay hypersensitivity reactions resulted from the toxic effect of carrageenan on macrophages (1).

Current immunologic theory explains the evolution of a delayed hypersensitivity reaction as a two-step process (17). The first event is the interaction between antigen and specifically sensitized small lymphocytes. This interaction may lead to release of chemotactic and other humoral factors which bring about local vascular changes, and an accumulation of macrophages. After a period of hours, the typical delayed, or tuberculin-type reaction, is observed (18).

The inhibition of delayed hypersensitivity reactions by carrageenan may be due to a direct toxic action of this compound on macrophages (1, 5). It may be, however, that its ability to activate the clotting, kinin, interrelated systems via its effect on Hageman factor may be responsible, at least in part, for this inhibitory action. Kellermeyer and

TABLE IV. The Effect of Carrageenan on Permeability Enhancing Activity of Human Plasma.

Mixture tested[a]	Permeability activity (mm)[b]
Normal plasma + buffer	2.4
+ carrageenan (3.3×10^{-1} mg/ml)	7.0
Hageman factor-deficient plasma + buffer	1.9
+ carrageenan (3.3×10^{-1} mg/ml)	2.0
Normal plasma + soybean trypsin inhibitor (0.25 mg/ml)	1.9
+ carrageenan (3.3×10^{-1} mg/ml) + soybean trypsin inhibitor[c] (0.25 mg/ml)	1.2
Carrageenan (3.3×10^{-1} mg/ml) + buffer	1.3

[a] Concentrations are for final volume.

[b] These numbers represent the average diameter of the blue spot developed after intradermal inoculation of 0.1 ml of test material into each of four guinea pigs preinjected with Pontamine sky blue.

[c] Soybean trypsin inhibitor added prior to carrageenan.

TABLE V. The Effect of Carrageenan on Kinin Activity Formation in Human Plasma.

Mixture tested	Kinin releasing activity[a]
Normal plasma + buffer + phenanthroline	0
+ carrageenan (3.3×10^{-1} mg/ml) + phenanthroline	+
Hageman factor-deficient plasma + carrageenan (3.3×10^{-1} mg/ml) + phenanthroline	0
Normal plasma + soybean trypsin inhibitor + phenanthroline + carrageenan (3.3×10^{-1} mg/ml)	0
Normal plasma + anti-Hageman factor globulin[b] + phenanthroline + carrageenan (3.3×10^{-1} mg/ml)	⅓
Normal plasma + buffer + phenanthroline + carrageenan (3.3×10^{-1} mg/ml)	+

[a] + = maximal contraction; 0 = no contraction; ⅓ represents a contraction 67% less than maximal.
[b] Normal plasma incubated with antiserum at 37° for 3 hr; carrageenan added for 15 min, then mixture was tested.

Warren recently found that the granuloma response to schistosome ova is inhibited when mice are pretreated with ellagic acid (an activator of Hageman factor) (13, 19). This granuloma model has many characteristics of a delayed hypersensitivity reaction (20). The suppression of two varieties of delayed hypersensitivity by materials which activate Hageman factor suggests the possibility that one of the Hageman factor-dependent sequences may play a role in delayed hypersensitivity reactions. Intravenous injection of ellagic acid resulted in kinin formation and kininogen depletion in rats (21). It thus seems possible that the effects of carrageenan and ellagic acid in delayed hypersensitivity reactions may in part be explained by a consumptive depletion of these factors. Further studies along these lines are now in progress.

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