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EFFECT OF ORALLY ADMINISTERED FOOD-GRADE CARRAGEENANS ON ANTIBODY-MEDIATED AND CELL-MEDIATED IMMUNITY IN THE INBRED RAT

S. NICKLIN and K. MILLER
Department of Immunotoxicology, British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey SM5 4DS, England

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Abstract—Experiments were performed to investigate the immunological consequences associated with the persorption of poorly degradable carregeenans from the diet. Using an inbred strain of rat it was demonstrated histochemically, by the carrageenan-specific Alcian blue staining technique, that small quantities of food-grade carrageenans given at 0.5% in drinking-water for 90 days could penetrate the intestinal barrier of adult animals. This apparently occurred via an intact mucosa in the absence of inflammatory or pathological lesions. The carrageenan was demonstrated in macrophage-like cells present within the villi and lamina propria of the small intestine. The oral administration of κ, λ or ι food-grade carrageenans did not affect local (biliary) or systemic antibody responses to gut commensal micro-organisms, or to orally-administered sheep erythrocytes. However, when sheep red blood cells were administered parenterally the ensuing anti-sheep red blood cell haemagglutinating antibody response was temporarily suppressed in carrageenan-fed rats. λ-Carrageenan and ι-carrageenan both significantly ($P \leqslant 0.01$ and $P \leqslant 0.05$, respectively) reduced the mid-phase (14–28 days) haemagglutinin response; κ-carrageenan (L100) was less effective but caused significant depression at day 21 ($P \leqslant 0.01$). Individual responses were, however, within the control range 35 days after sheep erythrocyte administration, thus indicating the temporary nature of this effect. Although carrageenan administration depressed the anti-sheep erythrocyte antibody response, it did not affect T-cell immune competence as measured by the popliteal lymph node assay for graft-versus-host reactivity.

INTRODUCTION

Carrageenan is the generic name given to high-molecular-weight (> 100,000 daltons) sulphated polygalactans derived from certain species of red algae. Three basic types can be isolated: κ, λ and ι. These have differing colloidal properties and are characterized by the type of intergalactan bonding and the degree of sulphation (Rees, 1972). Carrageenans are used extensively in the food industry as thickening, gelling and protein-suspending agents. Initially all grades of carrageenan were considered safe by the Food and Drug Administration (FDA, 1959) and were permitted for use as regulated food additives (FDA, 1969). However, subsequent reports implicated degraded forms of carrageenan (mol wt 30,000) in the induction of ulcers and metaplastic changes in the intestinal tract of a number of species of experimental animals (Fabian, Abraham, Coulston & Golberg, 1973; Sharratt, Grasso, Carpanini & Gangolli, 1970; Watt & Marcus, 1970). Although some of these findings were contested by other studies (Maillet, Bonfils & Lister, 1970; Sharratt, Grasso, Carpanini & Gangolli, 1971) the FDA ruled that food-grade carrageenans should have an average molecular weight exceeding 100,000 (FDA, 1972). However, it is known that carrageenans undergo some degree of hydrolysis at gastric pH (Stoloff, 1959; Glicksman, 1969) and a proportion of the lower-molecular-weight polygalactan units produced can apparently gain access to the body tissues by persorption (Pittman, Golberg & Coulston, 1976).

Whilst there is no evidence to suggest that the persorption of small quantities of carrageenan across the epithelial barrier poses an acute toxic hazard, carrageenan is biologically active in a number of physiological systems. A comprehensive review of the biological properties of carrageenans was conducted by Di Rosa (1972). Subsequently several authors have reported that carrageenans, when administered parenterally, markedly affect a number of normal immune responses. These studies present data that indicate the suppressive effect of carrageenans on macrophage function (Di Rosa, 1972; Fowler, Simpson & Thomson, 1980), and in addition demonstrate carrageenan-mediated suppression of both antibody production (Rumjanek, Watson & Šljivić, 1977; Thomson, Fowler & Pugh-Humphreys, 1979) and cell-mediated immune responses (Rumjanek & Brent, 1978; Sakemi, Kuroiwa & Nomoto, 1980). Furthermore, Bash & Vago (1980), demonstrated that the oral administration of carrageenan to rats resulted in a dose-dependent suppression of lymphocyte responsiveness to *in vitro* mitogenic stimulation by phytohaemagglutinin.

In view of the potent biological properties of carrageenan and its wide application in the food industry, a study of the effects of this hydrocolloid on *in vivo* immune parameters was considered particularly relevant. The following feeding studies were

designed firstly to determine whether components of food-grade carrageenan could gain access to body tissues, and secondly to consider the effect of these materials on local (biliary) and systemic immune responses against intestinal bacteria and orally and systemically administered test antigen. The effects of carrageenan on T-cell-mediated immune reactivity was also studied using a graft-versus-host assay.

EXPERIMENTAL

Animals and treatment. Specified-pathogen-free (grade 3) PVG strain male rats weighing 200–250 g were purchased from Olac 1976 Ltd (Bicester, Oxfordshire). They were maintained under standard conditions (temperature $22 \pm 3°C$, humidity $60 \pm 15\%$, 15 room-air changes/hr, natural 12-hr light/dark cycle) on Spratt's Laboratory Animal Diet No. 1 (Spratt's Patent Ltd, Surrey), and given water *ad lib.* Body weight and food and water consumption were recorded weekly. After 7 days' acclimatization the rats were randomized into groups and thereafter given normal drinking-water (control) or drinking-water containing 0.5% λ-carrageenan (Type CSP), 0.5% ι-carrageenan (type J) or 0.5% κ-carrageenan (type L100). At these levels the daily intakes of the carrageenans were 0.15–0.25 g/day. The food-grade carrageenans were supplied by Hercules Ltd (London) and were dissolved in tap-water by homogenization prior to use. In each group half the rats were sensitized to sheep red blood cells (SRBCs; Oxoid Ltd, Basingstoke, Hampshire), by ip injection of 10^9 SRBCs in 1 ml saline on days 55 and 62 of the feeding study. All groups received an oral challenge of SRBCs (10^{10} cells in saline by gavage) on days 76 and 83. Blood samples for serum-antibody analysis were taken from the retro-orbital sinus on days 62, 69, 76, 83 and 90. The serum samples were stored at $-20°C$ until assayed. In addition, bile samples for antibody analysis were obtained on day 90 by bile-duct cannulation under pentabarbitone anaesthesia (Sagatal, May & Baker Ltd, Dagenham, Essex) after which the animals were killed. *Post mortem*, Peyer's patches including a section of gut immediately proximal and distal to each patch, were taken from the proximal, middle and terminal regions of the small intestine and embedded in wax using standard techniques for light microscopy. Half the sections were stained with haematoxylin and eosin, and the rest were stained using the carrageenan-specific Alcian blue staining technique (Gangolli, Wright & Grasso, 1973).

Antibody determinations. The total immunoglobulin A (IgA) content of bile samples was determined using the Mancini radial immunodiffusion technique (Mancini, Carbonara & Heremans, 1965), with immunoabsorbent purified rat biliary IgA (250 μg/ml) used as a standard. Serum and bile samples were examined for the presence of antibodies with binding specificity for carrageenan, using an enzyme linked immunoassay technique (ELISA) in conjunction with microtitre plates treated with poly-L-lysine and coated with 10 μg carrageenan/well (Johnson & Thorpe, 1982). The anti-SRBC antibody activity of serum and bile samples was determined by a haemagglutination procedure (Hudson & Hay, 1976).

The agglutination assays were performed in Steralin round-bottomed microtitre plates (Linbro/Titertek, Flow Laboratories Ltd, Strathclyde, Scotland), using 25 μl serum or bile heat-inactivated at 56°C for 30 min and serially double-diluted in phosphate buffered saline (PBS; pH 7.2) 1/2 to 1/256. After adding 25 μl of 5% SRBCs in suspension to each well, the plates were incubated at 4°C and scored for agglutination after 18 hr. The end-point titre was taken as the concentration at which agglutination was just detectable.

The immune response to the gut commensal *Escherichia coli* was determined in a similar manner using formalin-fixed *E. coli* (10^{10} organisms/ml) in place of the SRBCs. Reactivity against total gut microflora was assessed using an indirect immunofluorescence procedure. Gut bacteria were obtained from an established rat gut flora chemostat culture system maintained in these laboratories. The bacteria were washed in PBS and adjusted to a concentration of 10^{10} organisms/ml. Six 5-μl volumes of bacteria were applied as single spots to the surface of clean microscope slides, the spots were air dried and then heat fixed. Serial dilutions (dilution range: undiluted–1/32) of the serum or bile under study were then applied to the bacterial spots and the slides were incubated for 1 hr at room temperature in a humid atmosphere. The slides were washed thoroughly with PBS and flooded with a 1/100 dilution of rabbit anti-rat secretory IgA antiserum (Nordic Laboratory, Maidenhead, Berkshire) in PBS. After a further 30-min incubation the slides were washed and flooded for 30 min with fluorescein-labelled sheep anti-rabbit immunoglobin antiserum (Wellcome Research Laboratories, Beckenham, Kent) diluted 1/20 in PBS. After the unbound antiserum had been washed off, the slides were sealed with coverslips and examined microscopically using UV light. The fluorescent antibody titre was recorded as the highest concentration of serum or bile at which no bacterial fluorescence was visible.

Assessment of T-cell immune competence. The graft-versus-host (GvH), popliteal lymph node assay described by Ford, Burr & Simonsen (1970) was used to assess the effect of orally administered carrageenans on T-cell reactivity.

Rats were exposed as above to ι-carrageenan (J), κ-carrageenan (L100) or λ-carrageenan (CSP) supplied by Hercules Ltd. In addition, other rats were given drinking-water containing 0.5% food-grade ι-carrageenan (MCC) or 0.5% degraded ι-carrageenan from Marine Colloids, Inc. (Springfield, NJ, USA). After 90 days the rats were killed and cell suspensions were prepared in PBS from the spleens and mesenteric lymph nodes of the control and exposed animals. The cells were counted, adjusted to 2×10^8 per ml in PBS and held on ice prior to transfer (viability $> 95\%$ by trypan blue dye exclusion). Suspensions from test or control donors were injected (0.1 ml) subcutaneously into the right hind footpad of anaesthetized (PVG × DA) F_1 hybrid recipients matched for sex and age (SPF grade 3; OLAC 1976 Ltd). The left footpad was injected with (PVG × DA) F_1 (self) cells to serve as a negative control. Seven days after injection, the rats were killed and the right and left popliteal lymph nodes were removed, trimmed free of any adhering fat and weighed to an accuracy of

Table 1. Effect of carrageenan on rat biliary immunoglobulin A (IgA) concentration and antigen specificity

Treatment*	Group	IgA concn (mg/ml)	Anti *E. coli* titre (log_2 dilution)	Anti gut-flora titre (log_2 dilution)
Control	1	2.325 ± 0.174	2.16 ± 0.30	2.60 ± 0.41
Control	2	2.225 ± 0.255	3.00 ± 0.25	2.60 ± 0.82
κ (CSP)	3	2.725 ± 0.137	2.00 ± 0.51	3.00 ± 0.49
κ (CSP)	4	2.625 ± 0.252	2.33 ± 0.42	2.20 ± 0.49
λ (L100)	5	2.450 ± 0.232	2.00 ± 0.36	3.20 ± 0.80
λ (L100)	6	2.800 ± 0.111	3.00 ± 0.44	3.20 ± 0.80
ι (J)	7	2.650 ± 0.164	1.83 ± 0.16	2.80 ± 0.37
ι (J)	8	2.850 ± 0.175	2.50 ± 0.34	4.00 ± 0.66

*Treated animals were exposed to 0.5% carrageenan in drinking-water for 90 days. Groups 2, 4, 6 and 8 were sensitized to sheep erythrocytes. All values are means ± SEM for six animals.

0.1 mg. Comparison of the weight of the test popliteal lymph node (PLN) to that of the control PLN gives a measure of the degree of cellular proliferation within the lymph node. This ratio is referred to as the proliferation index and is proportional to the degree of T-cell-GvH reactivity expressed by the transferred PVG cells (Ford *et al.* 1970).

Statistics. Where appropriate, data were analysed using Student's t test, values of $P \leqslant 0.05$ being taken as significant.

RESULTS

Rats that were maintained on normal diets and drinking-water containing carrageenan showed no signs of abnormal behaviour or appearance. They showed no significant difference from controls in body-weight gain or food and water consumption. Sections of intestines and Peyer's patches stained with haematoxylin and eosin revealed no abnormal histological features or pathological lesions attributable to treatment. However, the Alcian blue staining technique specific for carrageenan (Gangolli *et al.* 1973) revealed small numbers of cells positive for Alcian blue within the intestinal villi, lamina propria and basement-membrane lymphatics of the carrageenan-treated rats (Fig. 1). The chemical nature of the carrageenan administered had no apparent effect on the degree of uptake or the distribution within the intestinal tissues.

The feeding regimens did not modify either the rate of bile secretion (data not reported) or the total IgA concentration in the bile (Table 1). ELISA analysis of the 90-day serum and bile samples indicated an absence of antibodies with binding specificity for carrageenan, indicating that although carrageenans gained access to lymphoid tissues, a specific anti-carrageenan antibody response could not be demonstrated (data not shown). Antibody analysis of day-90 bile samples for humoral reactivity to intestinal bacteria demonstrated antibodies in equivalent amounts in all test and control samples (Table 1). Examination of day-90 serum samples for anti-bacterial antibody activity (Table 2) revealed that all animals previously sensitized to SRBCs possessed higher levels of anti-*E. coli* antibodies than the non-sensitized groups. A similar picture emerged when the same serum samples were analysed for antibodies with binding specificity for total gut bacteria using the indirect immunofluorescence assay, but since equivalent levels of antibody were detected in the pre-sensitized control rats the immunization effect is clearly unrelated to the feeding regimen and apparently relates to the parenteral administration of SRBCs.

The oral administration of SRBCs to non-sensitized animals on days 76 and 83 of the feeding regimens did not initiate a local (biliary) or systemic (serum) anti-SRBC response. Similarly the oral administration of SRBCs to animals presensitized to SRBCs by injection did not lead to any significant enhancement of the existing anti-SRBC response.

To investigate the possible immunomodulatory properties of the test materials, serum samples were taken from parenterally immunized rats on days 62, 69, 76, 83 and 90. Serum analysis for anti-SRBC haemagglutinating activity demonstrated that orally administered carrageenan modified the normal immune response profile to SRBCs injected ip (Table 3). λ-Carrageenan (CSP) and ι-carrageenan both significantly reduced the mid-phase (days 14–28 after injection) haemagglutinin response; κ-carrageenan (L100) was less effective but caused significant depression compared with controls on day 21 after injection. All responses were within the control range by day 35 after injection. Although carrageenan administration depressed the anti-SRBC antibody response, it did not effect T-cell immune competence as measured by GvH reactivity (Table 4).

DISCUSSION

These studies indicate that small quantities of food-grade carrageenans given in the drinking-water

Table 2. Effect of carrageenan on the level of rat serum antibodies specific for gut micro-organisms

Treatment*	Group	Anti *E. coli* titre (log_2 dilution)	Anti gut-flora titre (log_2 dilution)
Control	1	2.80 ± 0.58	1.80 ± 0.36
Control	2	4.40 ± 0.67	4.40 ± 0.39
κ (L100)	3	2.00 ± 0.31	1.80 ± 0.19
κ (L100)	4	5.20 ± 0.37	3.80 ± 0.37
λ (CSP)	5	2.00 ± 0.00	2.00 ± 0.31
λ (CSP)	6	4.60 ± 0.39	4.20 ± 0.85
ι (J)	7	1.80 ± 0.37	1.60 ± 0.50
ι (J)	8	4.80 ± 0.19	4.00 ± 0.70

*Animals were exposed to 0.5% carrageenan in drinking-water for 90 days.
Groups 2, 4, 6 and 8 were sensitized to sheep erythrocytes. All values are means ± SEM for six animals.

Table 3. Effect of carrageenan administration on the rat serum haemagglutinin response to sheep red blood cells injected ip

Day of Response*	Haemagglutination titre (log_2 dilution)			
	Control	ι (J)	λ (CSP)	κ(L 100)
7 (62)	5.80 ± 0.44	4.00 ± 0.93	4.80 ± 0.33	6.20 ± 0.18
14 (69)	6.20 ± 0.33	4.80 ± 0.44	4.40 ± 0.54**	5.60 ± 0.22
21 (76)	6.40 ± 0.22	4.40 ± 0.54**	4.20 ± 0.34**	5.40 ± 0.22*
28 (83)	6.20 ± 0.17	5.40 ± 0.34*	5.80 ± 0.17	5.80 ± 0.17
35 (90)	6.40 ± 0.36	5.40 ± 0.46	5.40 ± 0.22	6.00 ± 0.28

*Day of feeding study in parentheses. Treated animals were exposed to 0.5% carrageenan in drinking-water for 90 days.
Values are means ± SEM for six rats. Values marked with asterisks are significantly different (Student's t test) from the corresponding control value ($*P \leqslant 0.05$; $**P \leqslant 0.01$).

Table 4. Effect of carrageenans on rat T-lymphocyte immune competence as measured by graft-versus-host reactivity

Treatment of lymphocyte donors*	No. of recipients†	Proliferation index‡ (mean ± SEM)
Control	6	11.87 ± 1.49
ι (J)§	7	12.53 ± 1.05
λ (CSP)§	7	14.21 ± 2.00
κ (L100)§	7	11.49 ± 0.84
ι (MCC)§	6	14.39 ± 1.62
ι degraded (MCC deg)§	6	13.33 ± 2.04

*PVG rats.
†(PVG × DA) F_1 rats.
‡Proliferation index = (wt of test lymph node) ÷ (wt of contralateral control lymph node).
§Treated animals exposed to 0.5% carrageenan in drinking-water for 90 days.

were persorbed across the mucosal interface of the gut. This result confirms the observations of Pittman *et al.* (1976), who demonstrated that although the majority of high-molecular-weight carrageenans given orally were excreted, a small amount entered the body tissues. The present study demonstrated the route of carrageenan access to be via a population of cells within the intestinal villi and lamina propria. The final destination of the carrageenan and its subsequent fate remain to be determined. It is also not possible to conclude whether the histochemical method detected persorbed food-grade (high mol wt) carrageenan or degraded carrageenan produced by gastric hydrolysis of the parent food-grade molecules. Since persorption occurred in the absence of inflammatory or pathological changes it seems probable that the carrageenan-containing cells were fulfilling a normal role in sampling antigenic components from the intestinal lumen and transporting the material to the gut-associated lymphoid tissues. It is apparent from a number of studies that intestinal components readily gain access to the body across an intact mucosal surface via macrophage-like cells within the lamina propria and the subepithelial zone of Peyer's patches (Pugh, Macpherson & Steer, 1983; Sminia, Janse & Wilders, 1982). Indeed, evidence suggests that the persorption of intact proteins, for example, is a prerequisite for the induction of immune tolerance to these dietary components (Challacombe & Tomasi, 1980). The effects of poorly degradable macromolecules are less clear. The carrageenans were shown not to elicit an antibody response. This was possibly because carrageenans, like other polysaccharide molecules, are only weakly immunogenic; alternatively it may be that persorbed carrageenans, like proteins, induce specific tolerance in the immune system.

Antibody responses to the commensal gut microflora or to test antigen given orally were shown to be unaffected by the carrageenan treatment. These results support the histological findings, which indicated no apparent intestinal damage, since intraintestinal immunization to microflora or orally administered sheep erythrocytes has been shown to occur only under conditions associated with epithelial trauma or non-specific inflammation (Nicklin & Miller, 1983). However, the administration of carrageenans was shown to depress the antibody response to SRBCs administered systemically (ip). Although statistically significant suppression occurred during the early-to-middle phase of the response, antibody responses were within the control range 35 days (day 90 of the feeding study) after the administration of SRBCs. These results suggest that carrageenans delay rather than actively suppress the antibody response.

The anti-SRBC response is known to be T-cell dependent. Nevertheless, the GvH results clearly indicate that carrageenan does not affect T-cell competence. Macrophages also play an essential role in the induction of antibody responses. These cells appear to be responsible for the uptake, transport and processing of antigen as well as for the presentation of antigen to the antibody-producing B-cells. Since it is known that certain grades of carrageenan are cytotoxic to macrophages following *in vitro* or systemic exposure (Thomson *et al.* 1979), it is possible that the observed depression of the anti-SRBC response was due to a deficiency in antigen processing by macrophages rather than a depletion in immunocompetent cells. However, the assumption that all carrageenans are cytotoxic for macrophages has been challenged by some authors. Aschheim & Raffel (1972) demonstrated that antigen-laden macrophages obtained from animals previously injected with carrageenan could still stimulate antibody production in normal recipients. Similarly Simon & Jones (1975) were unable to demonstrate carrageenan-dependent toxicity in cultures of mouse peritoneal macrophages that had clearly ingested the material. These apparently conflicting results can, however, be partly resolved by considering the effect of carrageenan on the immunoregulatory activity of macrophages. Rumjanek *et al.* (1977) demonstrated that macrophages from carrageenan-treated mice could actively sup-

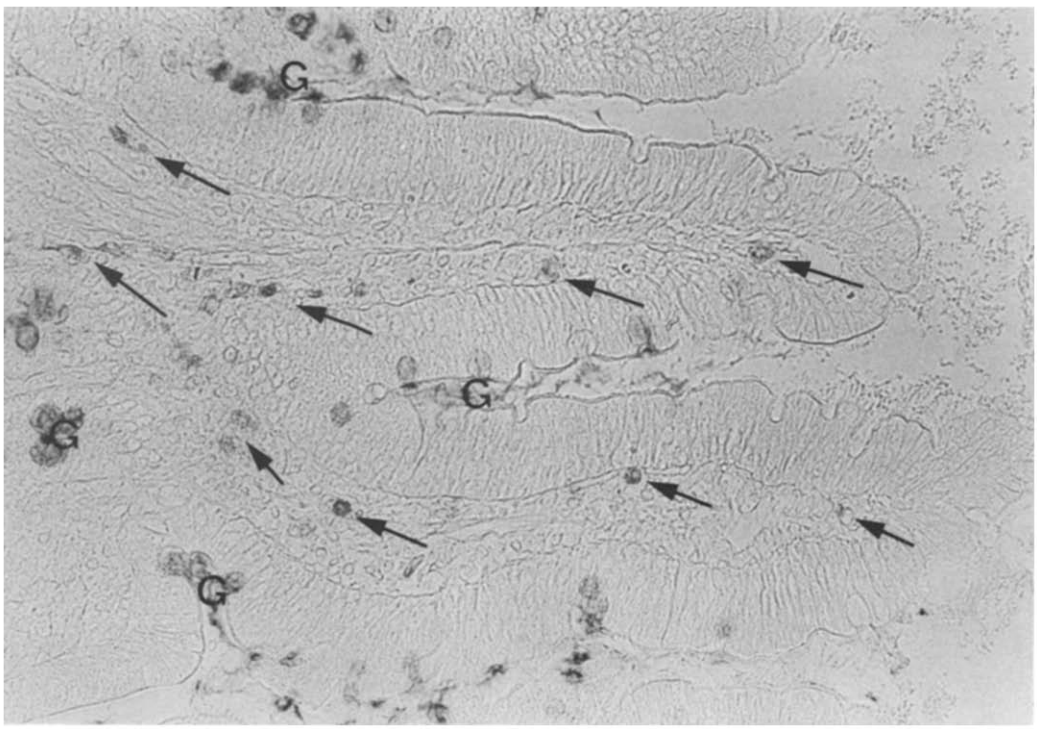

Fig. 1. Distribution of cells stained with Alcain Blue (indicated by arrows) within the subepithelial lymphatic space of duodenal villi in rats given 0.5% ι-carrageenan (type J) in drinking-water. G = Goblet cells counterstained using Neutral red.

press the antibody response of normal spleen cells to SRBCs. More recently Bash & Cochran (1980) demonstrated that macrophages stimulated *in vivo* with low doses of carrageenan produced a soluble inhibitor of T-lymphocyte proliferation. It is therefore also conceivable that the altered immune response observed in the present study may be due to the release of immunoregulatory factors from macrophages rather than through direct toxicity.

Studies are presently in progress to determine the effect of food-grade carrageenans on the secretory activity of cultured macrophages.

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REFERENCES

Aschheim L. & Raffel S. (1972). The immunodepressant effect of carrageenin. *J. Reticuloendothel. Soc.* **11,** 253.

Bash J. A & Cochran F. R. (1980). Carrageenan-induced suppression of T lymphocyte proliferation in the rat: *in vitro* production of a suppressor factor by peritoneal macrophages. *J. Reticuloendothel. Soc.* **28,** 203.

Bash J. A. & Vago J. R. (1980). Carrageenan-induced suppression of T lymphocyte proliferation in the rat: *In vivo* suppression induced by oral administration. *J. Reticuloendothel. Soc.* **28,** 213.

Challacombe S. J. & Tomasi T. B., Jr (1980). Systemic tolerance and secretory immunity after oral immunization. *J. exp. Med.* **152,** 1459.

Di Rosa M. (1972). Biological properties of carrageenan. *J. Pharm. Pharmac.* **24,** 89.

Fabian R. J., Abraham R., Coulston F. & Golberg L. (1973). Carrageenan-induced squamous metaplasia of the rectal mucosa in the rat. *Gastroenterology* **65,** 265.

FDA (1959). *Federal Register* **24,** 9368.

FDA (1969). *Federal Register* **26,** 94.

FDA (1972). *Federal Register* **37,** 15434.

Ford W. L., Burr W. & Simonsen M. (1970). A lymph node weight assay for the graft-versus-host activity of rat lymphoid cells. *Transplantation* **10,** 258.

Fowler E. F., Simpson J. G. & Thomson A. W. (1980). Histological and ultrastructural changes following carrageenan injection in the mouse. *J. Path.* **132,** 63.

Gangolli S. D., Wright M. G. & Grasso P. (1973). Identification of carrageenan in mammalian tissues: an analytical and histochemical study. *Histochem. J.* **5,** 37.

Glicksman M. (1969). *Gum Technology in the Food Industry.* 1st Ed. p. 222. Academic Press, New York.

Hudson L. & Hay F. C. (1976). *Practical Immunology.* 1st Ed. p. 125. Blackwell Scientific Publications, Oxford.

Johnstone A. & Thorpe R. (1982). *Immunochemistry in Practice.* 1st Ed. p. 252. Blackwell Scientific Publications, Oxford.

Maillet M., Bonfils S. & Lister R. E. (1970). Carrageenan: effects in animals. *Lancet* **II,** 414.

Mancini G., Carbonara C. O. & Heremans J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* **2,** 235.

Nicklin S. & Miller K. (1983). Local and systemic immune responses to intestinally presented antigen. *Int. Archs Allergy appl. Immun.* **72,** 87.

Pittman K. A., Golberg L. & Coulson F. (1976). Carrageenan: the effect of molecular weight and polymer type on its uptake, excretion and degradation in animals. *Fd Cosmet. Toxicol.* **14,** 85.

Pugh C. W., MacPherson G. G. & Steer H. W. (1983). Characterization of nonlymphoid cells derived from rat peripheral lymph. *J. exp. Med.* **157,** 1758.

Rees D. A. (1972). Shapely polysaccharides. The eighth Colworth Medal lecture. *Biochem. J.* **126,** 257.

Rumjanek V. M. & Brent L. (1978). Immunosuppressive activity of carrageenan for cell-mediated responses in the mouse. *Transplantation* **26,** 113.

Rumjanek V. M., Watson S. R. & Šljivić H. (1977). A re-evaluation of the role of macrophages in carrageenan-induced immunosuppression. *Immunology* **33,** 423.

Sakemi T., Kuroiwa A. & Nomoto K. (1980). Effect of carrageenan on the induction of cell-mediated cytotoxic responses *in vivo*. *Immunology* **41,** 297.

Sharratt M., Grasso P., Carpanini F. & Gangolli S. D. (1970). Carrageenan ulceration as a model for human ulcerative colitis. *Lancet* **II,** 932.

Sharratt M., Grasso P., Carpanini F. & Gangolli S. D. (1971). Carrageenin ulceration and ulcerative colitis. *Gastroenterology* **61,** 410.

Simon L. & Jones T. L. (1979). Re-evaluation of carrageenan cytotoxicity for macrophages. *J. Reticuloendothel. Soc.* **25,** 133.

Sminia T., Janse E. M. & Wilders M. M. (1982). Antigen-trapping cells in Peyer's patches of the rat. *Scand. J. Immun.* **16,** 481.

Stoloff L. (1959). Carrageenan. In *Industrial Gums, Polysaccharides and Their Derivatives.* Edited by R. L. Whistler & J. N. BeMiller. p. 83. Academic Press, New York.

Thomson A. W., Fowler E. F. & Pugh-Humphreys R. G. P. (1979). Immunopharmacology of the macrophage-toxic agent carrageenan. *Int. J. Immunopharmac.* **1,** 247.

Watt J. & Marcus R. (1970). Hyperplastic mucosal changes in the rabbit colon produced by degraded carrageenin. *Gastroenterology* **59,** 760.