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Intestinal uptake and immunological effects of carrageenan—current concepts

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Carageenans are a group of high molecular weight sulphated polygalactans which find extensive use in the food industry as thickening, gelling and protein-suspending agents. Although there is no evidence to suggest that the persorption of small amounts of carageenans across the intestinal barrier poses an acute toxic hazard, they are known to be biologically active in a number of physiological systems and extended oral administration in laboratory animals has been shown to modify both *in vivo* and *in vitro* immune competence. Whereas this effect could be attributed to carrageenan having a selective toxic effect on antigen-processing macrophages, additional studies suggest that macrophages can also influence immune responses by the timed release of immunoregulatory mediators. Evidence in support of this comes from *in vitro* studies which demonstrate that carrageenan-treated macrophages can, depending on conditions and time of administration, release either stimulatory or inhibitory factors. The former is known to be the immunostimulatory agent interleukin 1 (IL-1). The inhibitory factor, which is produced at an early stage following exposure to non-toxic doses of carrageenans, has yet to be formally identified but it is believed to be a prostaglandin because of its specific mode of action and short biological half-life.

At present it is impossible to relate these studies to the human situation. Although it is established that carrageenans can cross the intestinal barrier of experimental animals, there is no evidence to suggest that the limited uptake that may occur in man in any way interferes with normal immune competence. Nevertheless, increased exposure may occur in the neonate during weaning, and adults and children following allergic reactions and episodes of gastrointestinal disease. Further studies under such conditions now seem warranted in order to elucidate the possible immunological consequences which may be associated with enhanced uptake of carrageenans in vulnerable groups.

Introduction

Carrageenan is a generic term referring to a heterogeneous group of polysaccharides obtained by the aqueous extraction of certain species of red seaweed, in particular *Chrondrus crispus*, *Gigartina stellata* and various *Euchema* species. Today carrageenan is produced world-wide and is used extensively in the food industry as a thickening, gelling, stabilizing and protein-suspending agent. Processing steps used to recover carrageenan from seaweed vary considerably and are often closely guarded secrets. Cottrell and Baird (1980) state that the patent literature indicates that the process includes washing to remove soluble salts and debris before extraction with alkaline hot water. The carrageenan is then filtered and concentrated to about 3% by evaporation, prior to precipitation by alcohol. Alternatively, the extract is drum dried, but this yields a less consistent product.

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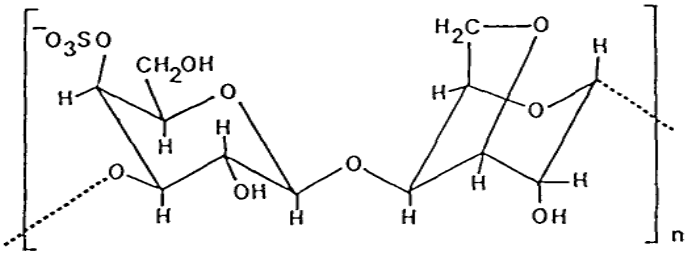
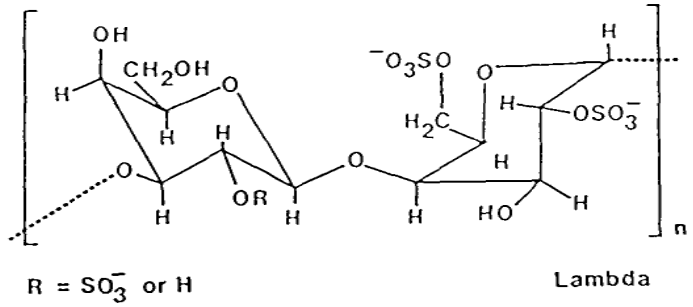
Crude carrageenan extracted from most species of seaweed can be fractionated further by the addition of KCl to produce a soluble fraction termed lambda (λ) and an insoluble fraction termed kappa (κ). Extracts from other selected species, most notably *Aghardhiella tenera* and *Euchema spinosum* sp. yield a third type, referred to as iota (ι). Intermediate salted fractions referred to as mu, nu, theta and xi can also be isolated, but these are usually converted into one of the above by chemical manipulation during processing (Towle 1973, Stancioff and Renn 1975). Current commercial usage is therefore restricted to kappa (κ), iota (ι) and lambda (λ). These have different colloidal and gelling properties and are characterized by the type of intergalactan bonding and their degree of sulphation (see below).

Structure

All carrageenans are long-chain high molecular weight (>100000 daltons) linear polymers of sulphated disaccharides, with regular substitution of some of the monosaccharide units to produce a complex repeating structure. The bonding





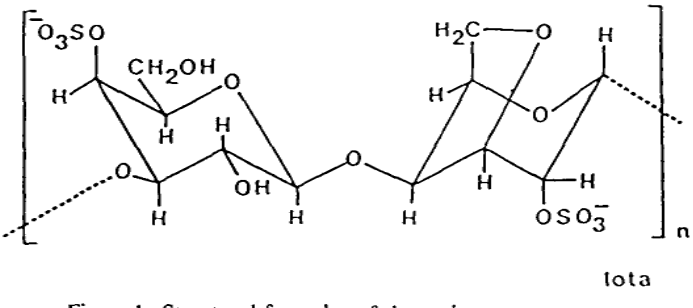


Figure 1. Structural formulae of the major carrageenans.

Table 1. Classification of the carrageenans.[a]

1,3-Linked unit	1,4-Linked unit	Name
D-Galactose 4-sulphate	3,6-Anhydro-D-galactose	kappa
	3,6-Anhydro-D-galactose 2-sulphate	iota
	D-Galactose 6-sulphase	mu
	D-Galactose 2,6-disulphate	nu
D-Galactose 2-sulphate	3,6-Anhydro-D-galactose 2-sulphate	theta
	D-Galactose 2-sulphate	xi
	D-Galactose 2,6-disulphate	lambda

[a]Adapted from Stancioff and Renn (1975).

between the monosaccharide units is alternatively β-D-(1,4) and α-D-(1,3) (Dolan and Rees 1965; Towle 1973). The idealized structure of kappa, lambda and iota carrageenan molecules are shown in figure 1 and a classification system for the carrageenans, based on the major monosaccharide constituents is shown in table 1.

Unlike many biological long-chain polysaccharides, carrageenans possess distinct secondary and tertiary structure. Kappa and iota carrageenans exist as right-hand, three-fold helices, which form double helices in the gel state (Rees 1972; Towle 1973). Lambda carrageenan is different in that the lack of a 3,6-anhydro bridge on the 1,4-linked units causes it to adopt a $^4C_1$ conformation, rather than the $^1C_4$ conformation exhibited by the 1,4-linked units of kappa and iota carragenan (IUPAC–IUB 1980) (see figure 1). Because of the change in the bond angles between the disaccharide units, lambda carrageenan forms a zig-zag ribbon structure and cannot gel.

Uses of carrageenan

Kappa and iota carrageenan form thermally reversible gels in water with lower concentrations yielding viscous solutions; lambda carrageenan exists only as viscous solutions (Towle 1973; FMC Corporation 1984). Carrageenans can also complex non-specifically with proteins both above and below their isoelectric point; this activity is particularly strong with respect to casein, and carrageenan is often used as a milk support in milk-based foods (Towle 1973). In addition to these properties, the rheological attributes of carrageenan solutions can be considerably modified by interactions with other polysaccharides or association with different cations.

More than 90 different culinary carrageenan preparations are produced world-wide (Cottrell and Baird 1980, IARC 1983; FMC Corporation 1984), and different carrageenan-based commercial food-additive mixtures can be blended to comply with an extremely broad range of customer requirements (Towle 1973). Carrageenan has been used for many years as an emulsifier, stabilizer and thickener in a variety of foods. It also serves as a suspension medium for flavouring and colouring agents in milk and other aqueous-based foods, and has many applications involving viscosity, gelation and improvement of 'mouth-feel'. The level of incorporation in various food products ranges from 0·005 to 1·2%, which equates to a daily intake per individual of 0–1·5 g depending on the choice of diet and total food consumed.

Biological studies

The biological activity of carrageenan has been examined in a number of studies using various routes of administration. However, not only is there no 'standardized'

preparation of carrageenan available, but also little information is given in most publications regarding the source, purity and even the class of carrageenan employed. Any evaluation of the biological effects of this material must therefore be tempered with the knowledge that different preparations may have different biological effects. There is obviously a real need to distinguish the active parameters of different carrageenans and the possibility of preparing a set of characterized carrageenan samples should be investigated at the international level.

For the purpose of this review, we shall concentrate on the effects of orally administered carageenans and in particular on studies relating to effects on the gut-associated immune system and immunologically related responses. Before discussing these in detail, however, it might be well to consider the salient features of the gastrointestinal immune system.

Gastrointestinal immune system

The epithelial lining of the gastrointestinal tract provides an extensive surface area for the absorption of digested food components necessary for the nutritional well being of the organism, yet it simultaneously presents a barrier to a vast number of itinerant microorganisms and exogenous antigens that continuously pass through the gastrointestinal tract as part of the daily dietary intake. However, whilst the bulk of undigested material appears to be quantitatively excluded, various pathogenic microorganisms and a small but immunologically significant amount of dietary components nevertheless gain access to the body tissues. Accordingly, the gut is richly endowed with gut-associated lymphoid tissue (GALT) both to generate immune effector functions and to monitor antigen uptake. The most important route of entry for intestinal antigen appears to be via the specialized epithelium cells overlying the Peyer's patches (figure 2). These structures are macroscopic aggregations of lymphoid tissue located immediately beneath the epithelium of the small intestine. Ultrastructurally Peyer's patches present a villus-free single-layered epithelium, overlying a dense lymphoid core. The unique feature of the epithelium is the presence of specialized columnar epithelial cells, referred to as M-cells in man (Owen and Nemanic 1978) and follicle-associated epithelial (FAE) cells in rodents (Bockman and Cooper 1973). These cells have the capacity to sample small amounts of material from the gut lumen and direct this material to the cells of the lymphoid core below. Other documented routes of uptake include persorption through and between the villi and non-specific uptake across an inflamed or damaged epithelium. The mesenteric lymph nodes in their turn receive and filter the cells and lymph collected in the intestine and conveyed to them by the afferent lymphatics. As these nodes lie downstream of both the *lamina propria* and the Peyer's patches, small lymphocytes, immunoblasts and macrophages are conveyed in significant numbers to this extra-intestinal site (Hall *et al.* 1987).

Antigen sampling of both soluble and particulate antigens is well documented (Nicklin 1987). The process occurs continuously and is considered to be a prerequisite for the initiation of local protective immunity (for a review, see Challacome 1987) and perhaps more importantly the induction of systemic antigen-specific immune tolerance. A special active transport mechanism has evolved to carry immunoglobin A (IgA) antibodies across the epithelium, while other antibodies and protein constituents of the tissue fluid are retained beneath the epithelium. At the mucosal surface IgA prevents the attachment and ingress of both pathogens and specific antigens. This state of tolerance involves a number of

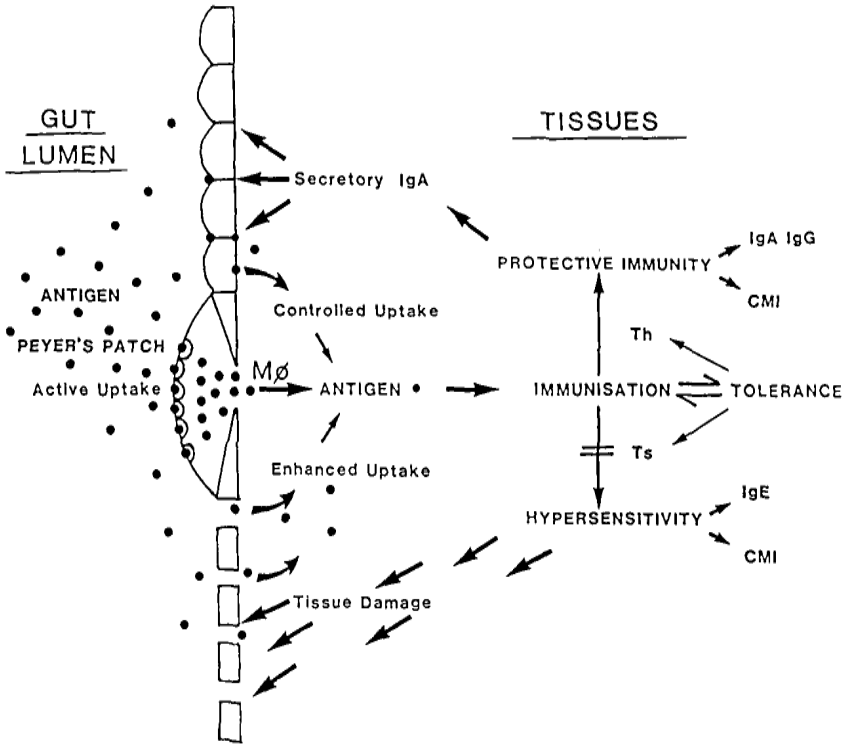


Figure 2. Schematic representation of antigen uptake across the intestinal epithelium and its interaction with the immune system.

mechanisms, the most important of which stem from the generation of antigen-specific suppressor/contra-suppressor cells. These lymphocytes block the initiation/elicitation of potentially damaging reactions and help maintain a *status quo* within the intestinal environment (for a review, see Challacombe and Tomasi 1987). Unfortunately, regulatory/defence mechanisms can be bypassed and the gut is often the initiation point for a range of untoward reactions including infections, immunopathological events and inflammatory and/or allergic-type reactions.

Intestinal uptake

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 (molecular weight 20 000) in the induction of ulcers and metaplastic changes in the intestinal tract of a number of species of experimental animals (Fabian *et al.* 1973; Sharratt *et al.* 1970, 1971, Marcus and Watt 1969, 1971, Watt and Marcus 1970). Herbivores such as guinea-pigs and rabbits were shown to be particularly sensitive, perhaps because of their herbivorous nature which is associated with a differing digestive capacity. In guinea pigs it was shown that the ulcerogenic capacity of the carrageenan was related to the ability of intestinal macrophages to endocytose the material and it was suggested that, following uptake, carrageenan caused lysosomal enzyme release and macrophage necrosis, prior to local tissue damage and ulceration (Abraham *et al.* 1974). Although some of the initial findings were contested by other studies (Maillet *et al.* 1970, Sharratt *et al.*

1971), the FDA ruled that food-grade carrageenans should have an average molecular weight exceeding 100 000 (FDA 1972).

The original studies of Marcus and Watt, however, also linked an undegraded carrageenan with pinpoint intestinal lesions in guinea pigs and rabbits. Subsequently studies using food-grade carrageenans in a variety of other species, including man, have failed to demonstrate any adverse effects, however. The consensus of scientific opinion therefore remained that whereas undegraded carrageenan may produce inflammatory lesions within the gastrointestinal tract of certain herbivores, omnivores were resistant.

Later controversy arose from a letter to *The Lancet* from Marcus and Watt (1980), in which they proposed that carrageenan might pose a carcinogenic hazard to man. This proposition stemmed from two sources, firstly the work of Wakabayashi *et al.* (1978) in Japan, who produced colorectal tumours in rats by the administration of 5 or 10% of degraded carrageenan in the diet, or 5% (w/v) of aqueous degraded carrageenan as the sole drinking fluid, and secondly a study performed in the USA using perhaps, more importantly, a native carrageenan. In the American study (Watanabe *et al.* 1978), groups of rats were maintained on a semi-synthetic diet with or without (15%) native carrageenan. Groups of rats additionally were treated with two classical carcinogens, namely azoxymethane and methylnitrosourea, which produce a variety of tumours in the rat. Carrageenan given at this high dietary level inhibited growth and produced chronic inflammatory changes in the large intestine but more importantly markedly enhanced the effect of both carcinogens. The controls receiving only carrageenan, however, presented no tumours and both Wantanabe *et al.* (1978) and more recently Mori *et al.* (1984) failed to associate carrageenan *per se* with either mutagenicity or genotoxicity. In response to the existing literature. Therefore, the IARC (1983) concluded that whereas degraded carrageenan should be viewed with suspicion, high molecular weight native carrageenan had no case to answer.

It is important to note that in the above studies carrageenan was given as a powder in the diet at up to 15% and a dry powder will interact with water within the gastrointestinal tract, causing dehydration and caecal enlargement. Such non-physiological exposure has also been shown to be associated with an altered caecal microflora and changes in microbial enzyme activity (Mallett *et al.* 1985).

Although native carrageenans are not ulcerogenic, trace amounts of this molecule have been shown to cross the intestinal barrier. Sharratt *et al.* (1970, 1971) reported that when native carrageenan 'labelled' with iron (III) was administered orally to guinea pigs, particulate Pearls' positive material could be demonstrated within *lamina propria* cells and the sub-epithelial macrophages of the caecum. Likewise, using the Alcian blue staining technique we have demonstrated the presence of carrageenan in the villus and *lamina propria* macrophages of rats given 0·5% carrageenan in their drinking water (Nicklin and Miller 1984). More recently, we confirmed and extended these observations using ^{3}H-labelled carrageenan and demonstrated the uptake of very low concentrations of radioactive label. Absorption occurred via the Peyer's patches and caecal lymph node, with the lymphatic drainage from these organs leading to the subsequent accumulation of radioactivity within the mesenteric lymph node (Baker 1986, Nicklin *et al.* 1988).

Uptake occurred in the absence of inflammatory or pathological changes and seemed consistent with the concept that the specialized cells present within the Peyer's patches and caecal lymphnode, sample carrageenan from within the

intestinal lumen and provide transport to the mesenteric lymph node, presumably for presentation to the immune system.

It has been suggested that the presence of lower molecular weight fragments in food-grade carrageenan or its possible generation following food processing or gastric hydrolysis may be hazardous to health (Marcus and Watt 1980, Ekström 1985). Although there is no evidence in man to suggest that intestinal uptake in any way poses a toxic hazard, carrageenans are physiologically active molecules and following systemic administration have been demonstrated to influence a number of biological systems, including blood clotting, the complement system, the inflammatory process and various aspects of the immune response (for reviews, see Di Rosa 1972 and Thompson and Fowler 1981).

Immunological consequences

The fate and effects of carrageenan *in vivo* are difficult to predict. As stated above, carrageenans are biologically active in a number of experimental systems and several workers have reported that systemically administered carrageenans can markedly affect a number of normal immune functions. However, as stated earlier, the lack of standardized carrageenan samples makes it difficult to compare directly the results obtained by different workers since different types and grades of carrageenan have often been used.

If introduced into a suitable site, such as the plantar surface of a rat's paw, the pleural cavity or a subcutaneous air bleb, carrageenan will induce an inflammatory response. In this response, oedema is followed by the migration of cells, mostly polymorphonuclear leucocytes, and in some sites the eventual formation of a granuloma (Di Rosa 1972). Other studies have demonstrated that systemic administration is associated with increased allograft survival. Rios and Simmons (1972) reported that histo-incompatible skin grafts showed extended survival on mice treated i.p. with kappa carrageenen, unless the mice were also treated with poly(2-vinylpyridine *N*-oxide) (PVNO), a lysosome-stabilizing agent. They concluded that the immunosuppression as evidenced by loss of host versus graft responses was mediated by macrophage cytotoxicity.

In vivo administration of carrageenan has been associated with a reduction in primary cytotoxic lymphocyte (CTL) responses to allogeneic cells and, conversely, an augmentation of the secondary CTL response (Sakemi *et al.* 1980). This differential effect was considered to be attributable to the differences in the macrophage requirement by lymphocytes in maturation or sensitization processes. The augmentation of the secondary response could also be due to macrophage injury caused by carrageenan, resulting in prolonged retention of injected allogeneic cells in the host and persistent antigenic stimulation of T cells (Yung and Cudkowicz 1977). Treatment of experimental animals with carrageenan also led to the inhibition of delayed hypersensitivity reactions (Bice *et al.* 1971, Schwartz and Catanzaro 1973). However, when carrageenan and antigen were administered together, the carrageenan acted as an adjuvant and has been shown to be an effective as Freund's complete adjuvant in potentiating the delayed-type hypersensitivity response to lysozyme (Mizushima *et al.* 1974).

In the main, however, there is general agreement that animals treated with carrageenan administered via the intraperitoneal route have a significantly reduced capacity to mount primary antibody responses against T-cell-dependent antigents (Ascheim and Raffel 1972, Thomson *et al.* 1976, Ishizaka *et al.* 1977, Rumjanek

et al. 1977). Antibody responses to T-cell-independent antigens are either unaffected (Ishizaka *et al.* 1977, Wong and Herscowitz 1979) or likewise suppressed (Chaouat and Howard 1976).

More importantly, Bash and Vago (1980) and Cochran and Baxter (1984) demonstrated that the oral administration of native carrageenan to rats also resulted in a dose-dependent suppression of lymphocyte responsiveness *in vitro*. The later results in particular are in accordance with more recent studies performed at BIBRA, which also demonstrated that the intestinal persorption of small amounts of orally administered food-grade carrageenan was associated with depressed systemic humoral immunity against a heterologous T-cell-dependent antigen (Nicklin and Miller 1984, Baker 1986, Nicklin *et al.* 1988).

As T-cell responses, as judged by a popliteal lymph node assay for graft versus host reactivity, remained unchanged during these studies (Nicklin and Miller 1984), we postulated that altered humoral immunity was the result of modified antigen processing by macrophages rather than a direct effect on lymphocytes. This view is supported by studies which demonstrated that carrageenans depressed some responses (Catalona *et al.* 1978, Catanzaro *et al.* 1971, Pugh-Humphreys and Thomson 1979, Rumjanek and Brent 1978) but enhanced others (Richou *et al.* 1968, Turner and Higginbottom, 1979), including the initiation of *de novo* reaginic antibody production against associated proteins (Nicklin *et al.* 1985) and haptens (Nicklin and Miller 1985).

These studies led to the suggestion that macrophages exposed to carrageenan may modify immune responses by the timed release of specific immunoregulatory products. Evidence in support of this possibility came from *in vitro* studies which demonstrated that carrageenan-treated macrophages could, depending on the conditions and time of administration, release either stimulatory and/or inhibitory factors. The former was shown to be the immunostimulatory agent interleukin 1 (IL-1) (Baker 1986). This is particularly noteworthy as IL-1 has been shown to be released from macrophages by other adjuvants (Oppenheim and Gerry, 1983). The inhibitory factor, which was shown to be produced at an early stage following exposure to non-toxic doses of carrageenan, has yet to be formally identified but it is believed to be a prostaglandin because of its mode of action and short biological half-life.

Prostaglandins have been shown to inhibit effectively a range of *in vitro* immune responses, including humoral antibody production (Webb and Nowowiejski 1977), mitogen-induced lymphocyte transformation (Rao *et al.* 1979), lymphocyte-mediated cytolysis (Henney *et al.* 1972) and the production of leukocyte (Lomnitzer *et al.* 1976) and macrophage migration inhibitation factor (Gordon *et al.* 1976). Further evidence directly implicating prostaglandins in the role of inhibitory immunoregulatory molecules is based on the ability of inhibitors of prostaglandin synthesis to enhance antibody production (Webb and Nowowiejski 1977) and mitogenicity responses (Vosixa and Thies 1979). Bash & Cochran (1980) also reported that a suppressor factor produced by incubating rat spleen cells with carrageenan was inhibited by indomethacin, a prostaglandin synthetase inhibitor. In reviewing these studies, it is possible to put forward explanations for certain aspects of the immunoregulatory properties of carrageenan. It would appear that the adjuvant properties of carrageenan stem from its ability both to act as a protein carrier and to elicit enhanced IL-1 release by antigen-presenting macrophages. This in turn would induce preferential amplification of T-helper cells which, if triggered

at an appropriate time, would result in an enhanced immune response including the elicitation of reaginic antibody production.

The immunodepressive effects observed when carrageenan is given orally or systemically prior to antigen is more difficult to explain, but could involve carrageenan-induced IL-1 dependent expansion of a T supressor cell population, the release of inhibitory prostaglandin molecules and/or direct macrophage toxicity. These effects are, of course, not necessarily mutually exclusive, and in view of the previous observations it seems likely that the final outcome with regard to the immune system must be influenced by both the dose of carrageenan administered and the conditions of exposure.

Conclusions

Summarizing the animal studies considered in this review it appears probable that the ulceration phenomenon associated with carrageenans is restricted to herbivores that either possess a special ability to absorb carrageenan or present intestinal tissues that are particularly sensitive to the biological effects of this material.

With respect to absorption, both rat and guinea-pig studies indicate that a trace proportion of orally administered food-grade carrageenan can gain access to the body tissues. In the rat the carrageenan was cell (presumed macrophage)-associated and uptake occurred in the absence of inflammation or pathological change. Although the actual molecular weight of the material that enters the cells is open to speculation, its presence is consistent with the normal process of antigen sampling that occurs constantly within the mammalian gastrointestinal tract. Whereas this clearly poses no acute hazard, carrageenans are biologically active molecules and long-term administration in rats is associated with reduced humoral responsiveness to a test antigen challenge. Additional *in vitro* studies indicate that this effect may be mediated via the release of immunoregulatory agents from carrageenan-containing macrophages.

At present it is impossible to relate these studies to the human situation. Although it is established that uptake of high molecular weight material occurs across the human gut, there is no evidence to suggest that the limited uptake of carrageenan that may occur in man in any way interferes with normal immune competence. Nevertheless, increased exposure may occur during allergic reactions and in episodes of gastrointestinal disease.

Physiological experimental studies to date have all been performed in adult animals with an intact gastrointestinal tract and little work has been undertaken on young animals during the postnatal and weaning phases. The young of both man and animals have poorly developed gut-associated lymphoid tissue at birth and more permeable epithelial barriers. Further studies under such conditions would elucidate the possible immunological consequences which may be associated with enhanced uptake of carrageenan in vulnerable groups.

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