Br. J. exp. Path. (1976) **57**, 455

TOXICITY OF VARIOUS CARRAGEENANS IN THE MOUSE

A. W. THOMSON AND C. H. W. HORNE

From the Department of Pathology, University Medical Buildings, Foresterhill, Aberdeen AB9 2ZD

Received for publication March 15, 1976

Summary.—Carrageenan toxicity was found to vary according to the biochemical nature and source of material injected. Ischaemic lesions of body extremities (acronecrosis) were produced by some but not all preparations. Histological examination of these sites and of both recent and old organ lesions (especially of liver and kidney) confirmed that the underlying pathology was disseminated intravascular coagulation. Evidence was also obtained that carrageenans are hepatotoxic.

CARRAGEENANS are high molecular weight sulphated polygalactans obtained from marine algae. They have a variety of biological effects (reviewed by Di Rosa, 1972) including induction of acute and chronic inflammatory responses, activation of Hageman factor and inhibition of the CI component of complement. In addition, however, there is evidence that carrageenan is selectively toxic to macrophages (Allison, Harington and Birbeck, 1966; Catanzaro, Schwartz and Graham, 1971; Thomson *et al.*, 1976*a*) and recent work has shown that it is a potent immunosuppressive agent in laboratory animals (Bice *et al.*, 1971; Aschheim and Raffel, 1972). Furthermore, it has been shown that combined carrageenan and conventional drug therapy is superior to azathioprine and promethazine treatment alone (Calne, Wall and Wilkins, 1975, 1976) in the prevention of renal allograft rejection in dogs. It would appear therefore, that carrageenan might be useful as an adjunct to conventional immunosuppressive therapy.

In this paper we should like to draw attention to the relative toxicity of various carrageenans. Survival rates in mice treated with various carrageenans have been determined and the nature of the pathology established over a period of 24 weeks.

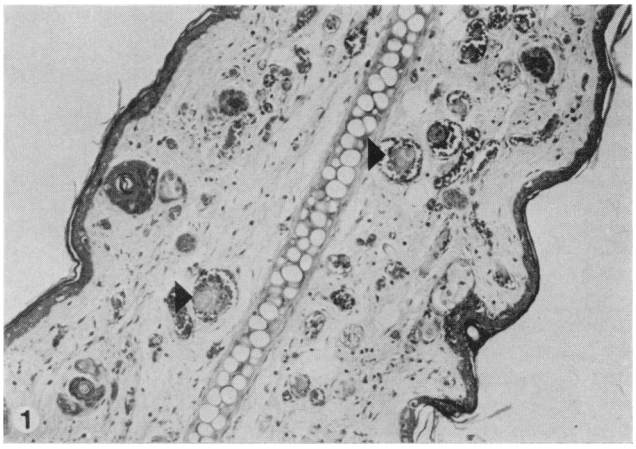
MATERIALS AND METHODS

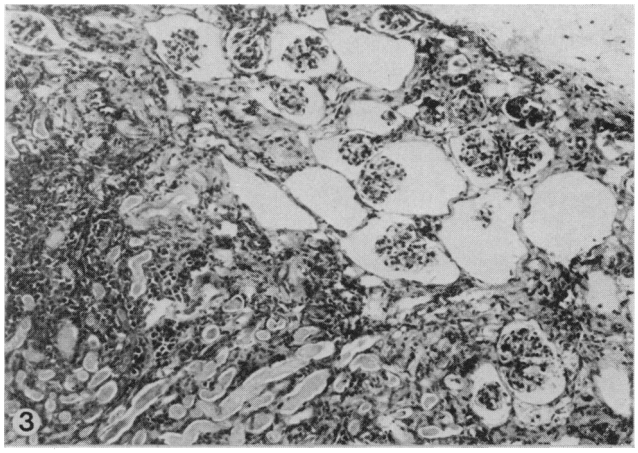
The carrageenans used were obtained from Sigma, London (uncharacterized potassium carrageenan), Marine Colloids Inc., Springfield, New Jersey (*kappa* and *lambda*) and from Dr F. B. Williamson, Biochemistry Department, Aberdeen University (*iota*). They were dissolved in boiling 0·85% phosphate-buffered saline (PBS) pH 7·2, sterilized by membrane filtration at 45–50° then injected i.p. in a volume of 0·2 ml. Closed colony-bred female LACA mice, 12–14 weeks of age, weighing 20–25 g and receiving a commercial diet and tap water *ad libitum* were used throughout.

Histological examination was performed on tissue fixed in 10% neutral buffered formalin. Paraffin sections were cut at 5 μm and stained with haematoxylin and eosin or Martius-Scarlet-Blue (MSB) stain for fibrin (Lendrum *et al.*, 1962).

RESULTS

The most obvious effect of i.p. injection of 1–25 mg potassium carrageenan was the appearance within 24 h of acronecrosis. All animals showed ischaemic necrosis of the distal portion of the tail. Doses of 5 mg or above gave rise to similar lesions of the ear margins, nose and limb digits, the incidence and extent of tissue damage being dose-dependent. The histological nature of the pathology was determined in separate groups of 5 animals given 1, 5, 10 and 25 mg of potassium carrageenan. Examination of subcutaneous tissue from the above sites showed numerous fibrin thrombi in small vessels (Fig. 1).





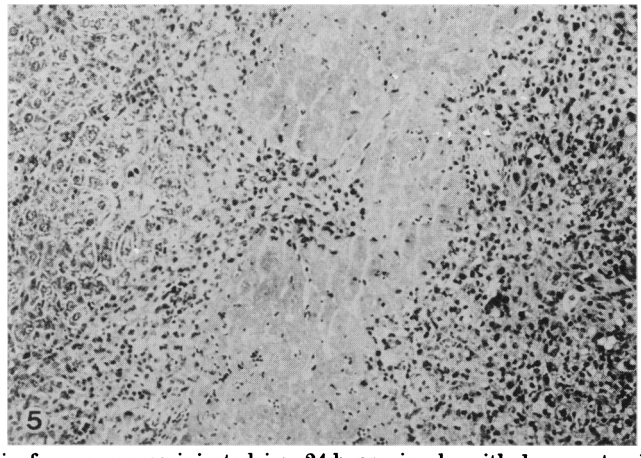


FIG. 1.—Ear margin from a mouse injected i.p. 24 h previously with 1 mg potassium carrageenan (Sigma). Fibrin thrombi (arrowed) are visible in small vessels. H and E. × 95.

FIG. 3.—Kidney from a mouse injected 24 weeks previously with 10 mg potassium carrageenan (Sigma). There is gross reduction in cortical thickness, fibrosis, focal chronic inflammatory cell infiltrate, tubular dilatation with protein casts. H and E. × 95.

FIG. 5.—Liver from a mouse injected 48 h previously with *lambda* carrageenan. A prominent area of necrosis can be seen in the liver parenchyma. H and E. × 95.

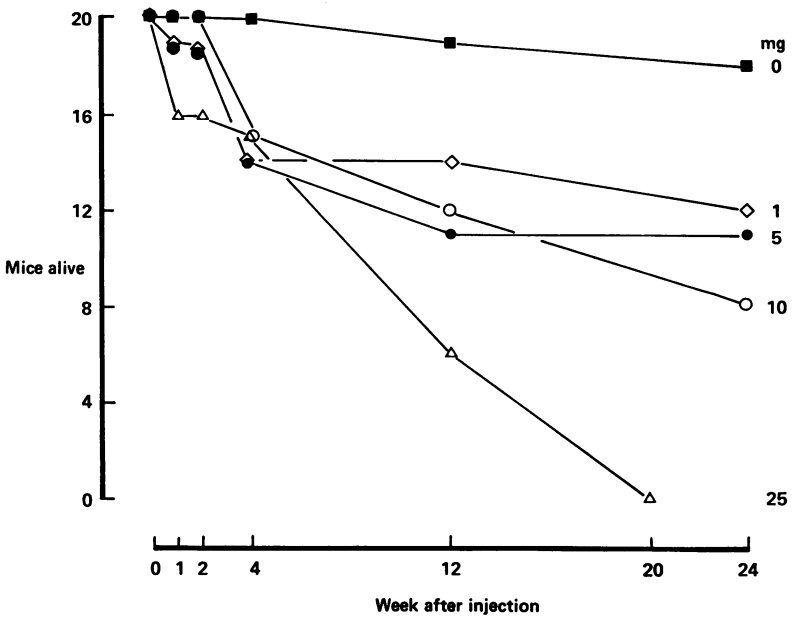


FIG. 2.—Effect of various doses of potassium carrageenan (sigma) on the survival of mice over a 24-week period. (■) phosphate-buffered saline (◇) 1 mg, (●) 5 mg, (○) 10 mg, (△) 25 mg.

Examination of the internal organs at 96 h in the various groups showed randomly distributed areas of necrosis in the liver and occasional fibrin thrombi were noted in liver, kidney and lung vessels, especially in those mice receiving 25 mg of potassium carrageenan. Regeneration of hepatocytes indicating minor degrees of liver damage was also noted in all animals receiving 10, 5 or 1 mg. The only other finding of note was striking splenomegaly in all mice. There was a marked increase in the amount of white pulp with prominent extramedullary haemopoiesis in the red pulp, megakaryocytes being particularly prominent.

The mortality rates over a 24-week period in groups of 20 animals receiving a single i.p. injection of various doses of potassium carrageenan are shown in Fig. 2. Clearly, in comparison with PBS injected mice, the mortality rate was increased in each group of mice receiving this preparation. The incidence of deaths amongst animals receiving 1–10 mg was greater during the first 4 weeks after injection than over the ensuing 20 weeks. At the highest dose used (25 mg), 75% of mice were alive 1 month after injection; however, the mice continued to die and no mice in this group survived after 20 weeks of the study. The only significant finding in autopsies performed on these animals was irregular scarring and gross reduction in size of one or both kidneys. Histological examination of these organs revealed irregular areas of fibrosis with loss of glomeruli, reduction in the number of tubules and dilatation of surviving tubules (Fig. 3).

The toxicity of *kappa*, *lambda* and *iota* carrageenans was evaluated in groups of 20 mice receiving a single i.p. injection of 5 mg. Acronecrosis developed only in animals receiving *kappa* carrageenan. Further, although neither *kappa* nor *iota* produced fatalities over the first 4 weeks, *lambda* carrageenan had a dramatic effect, 70% of the treatment group dying within one week (Fig. 4). Internal damage was assessed in separate groups of 5 animals treated with the various preparations.

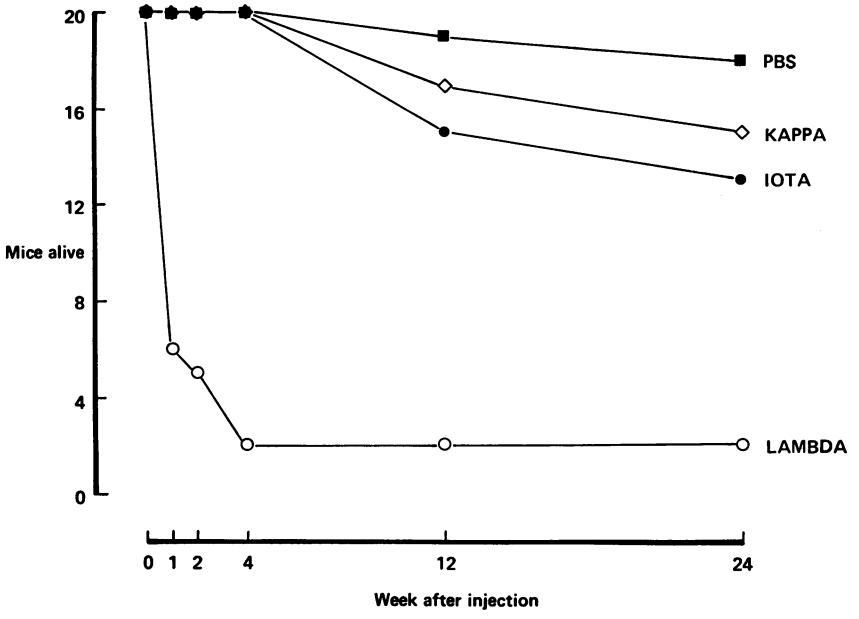


FIG. 4.—Effect of various carrageenans (5 mg) on the survival of mice over a 24-week period. (■) phosphate-buffered saline, (◇) *kappa*, (●) *iota*, (○) *lambda*.

At autopsy 96 h after treatment, the only macroscopic evidence of tissue damage was in the *lambda* group, pale areas being noted in the liver. Histological examination of spleen, lung and kidney from all animals in these groups showed evidence of minimal liver damage, and marked reactive changes in the spleen identical to those seen in the potassium carrageenan group. Areas of necrosis in the liver parenchyma (Fig. 5) were noted in the *lambda* group especially in these animals dying within 24 h. Autopsy of *kappa*, *lambda* and *iota* carrageenan treated survivors at 24 weeks revealed no obvious pathology and no histology was taken.

DISCUSSION

Our findings suggest that carrageenans induce disseminated intravascular coagulation leading, in some cases, to areas of infarction both in internal organs and extremities. The existence of thrombi in small vessels, which supports this claim, confirms the observation of Bice *et al.* (1972). Carrageenans are known to activate Hageman factor (Schwartz and Kellermeyer, 1969) which, in turn, results in the release of kinins and eventually leads to thrombosis. The gross kidney damage observed several weeks after carrageenan injection is almost certainly due to ischaemia following intravascular coagulation. The marked toxicity of *lambda* carrageenan may simply be attributable to ischaemia but in view of the diffuse nature of the liver damage with all carrageenans it seems probable that these substances are cytotoxic to hepatocytes. Hepatotoxicity is a property shared with another macrophage toxic agent, silica (Allison *et al.* 1966). Interpretation of the toxicology of carrageenans would be illuminated by evaluation of their distribution patterns and clearance rates *in vivo*. Such studies are dependent on production of labelled pure polysaccharide preparations.

Each carrageenan preparation increased the cellularity of the white pulp or thymus dependent lymphocyte area of the

spleen. However, carrageenan does not appear to stimulate T cell function *in vivo* (Thomson and Horne, 1975) but is known to initiate the recruitment or trapping of lymphocytes within lymphoid organs, a property of several known immunological adjuvants (Frost and Lance, 1973).

Lambda and *iota* carrageenans are particularly effective suppressants of antibody production in the mouse (Thomson *et al.* 1976*b*) and since, as we have shown, *iota* carrageenan is relatively non-toxic, it has perhaps the greatest prospective value in analysis of the role of the macrophage in induction and expression of immune reactivity. Further, *iota* carrageenan would appear to be the preparation of choice in the evaluation of this sulphated polysaccharide either as an immunosuppressant in its own right, or as an adjunct to suppressive therapy.

We are grateful to Mr A. McKinnon and Mrs A. Masson for preparing the tissue for histological examination, to the staff of the Animal Department, Foresterhill, for management of the animals, to the Department of Medical Illustration, Aberdeen Medical School for preparation of the figures and to Miss Annabel Mackay for preparing the manuscript.

REFERENCES

ALLISON, A. C., HARINGTON, J. S. & BIRBECK, M. (1966) An Examination of the Cytotoxic Effects of Silica on Macrophages. *J. exp. Med.*, **124**, 141.

ASCHHEIM, L. & RAFFEL, S. (1972) The Immunodepressant Effect of Carrageenan. *J. reticuloendothel. Soc.*, **11**, 253.

BICE, D., GRUWELL, D. G., SALVAGGIO, J. E. & HOFFMAN, E. C. (1972) Suppression of Primary Immunization by Carrageenan—A Macrophage Toxic Agent. *Immunol. Commun.*, **1**, 615.

BICE, D., SCHWARTZ, H. J., LAKE, W. W. & SALVAGGIO, J. (1971) The Effect of Carrageenan on the Establishment of Delayed Hypersensitivity. *Int. Arch. Allergy*, **41**, 628.

CALNE, R. Y., WALL, W. J. P. & WILKINS, D. C. (1975) Inhibition of Rejection of Canine Renal Allografts by Treatment with Sulphated Polysaccharides, Promethazine Hydrochloride and Azathioprine. *IRCS Med. Sci.*, **3**, 556.

CALNE, R. Y., WALL, W. J. P. & WILKINS, D. C. (1976) The Individual and Combined Roles of Carrageenan, Promethazine Hydrochloride and Azathioprine as Immunosuppressants in Dogs with Renal Allografts. *IRCS Med. Sci.*, **4**, 19.

CATANZARO, P. J., SCHWARTZ, H. J. & GRAHAM, R. C. (1971) Spectrum and Possible Mechanism of Carrageenan Cytotoxicity. *Am. J. Path.*, **64**, 387.

DI ROSA, M. (1972) Biological Properties of Carrageenan. *J. Pharm. Pharmac.*, **24**, 89.

FROST, P. & LANCE, E. M. (1973) The Relation of Lymphocyte Trapping to the Mode of Action of Adjuvants. In *Immunopotentiation.* Ciba Foundation Symposium 18. Amsterdam: Elsevier. Excerpta Medica.

LENDRUM, A. C., FRASER, D. S., SLIDDERS, W. & HENDERSON, R. (1962) Studies on the Character and Staining of Fibrin. *J. clin. Path.*, **15**, 401.

SCHWARTZ, H. J. & KELLERMEYER, R. W. (1969) Carrageenan and Delayed Hypersensitivity II. Activation of Hageman Factor by Carrageenan and its Possible Significance. *Proc. Soc. exp. Biol. Med.*, **132**, 1021.

THOMSON, A. W. & HORNE, C. H. W. (1975) Failure of Carrageenan to Affect Graft-versus-host-Reactivity in the Rat. *Transplantation*, **20**, 435.

THOMSON, A. W., WILSON, A. R., CRUICKSHANK, W. J. & JEFFRIES, A. H. (1976*a*) Evidence for a Selective Cytotoxic Effect of Carrageenan on Cells of the Immune System *In Vivo* and *In Vitro*. *Experienta*, **32**, 525.

THOMSON, A. W., WILSON, A. R., CRUICKSHANK, W. J. & HORNE, C. H. W. (1976*b*) Evaluation of Carrageenan as an Immunosuppressive Agent and Mediator of Intravascular Coagulation. *Biomedicine*, **24**, 102.