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Lysosomal Dysfunction in Colonic Submucosal Macrophages of Rhesus Monkeys Caused by Degraded Iota Carrageenan[1] (38996)

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The carrageenans represent a heterogeneous group of polygalactans extracted from seaweed, possessing variable degrees of sulfation. These polygalactans are used as antipeptic agents (C16) or as a dispersant in the food industry (HMR) (1). Administration of C16 in the drinking water causes cecal or colonic ulceration in guinea pigs, rabbits, and monkeys (2–5), squamous metaplasia of the anorectal junction of the rat (6), and storage of the macromolecule in Kupffer cell lysosomes of the monkey (1). The food grade carrageenans (HMR) are without adverse side effects when administered to guinea pigs, rats, monkeys, or pigs (1–4). Observations on the specific functional alterations from the submucosal macrophages of rhesus monkey colon are presented in this report.

Materials and methods. C16, a degraded iota carrageenan (Mw. Ca. 20,000), derived from *Eucheuma spinosum* by acid hydrolysis and peroxide precipitation (provided by Laboratoires Glaxo, Paris, France) (Batch L4006) was administered in a 2% solution, for 10 weeks as the sole source of drinking water to six rhesus monkeys (*Macaca mulatta*), three males and three females weighing 3–3.5 kg. Two animals from this group (one male and one female) were taken off medication after 10 weeks and allowed to recover for 24 weeks. HMR, an undegraded kappa and lambda carrageenan mixture (Mw. Ca. 800,000), derived from *Chondris crispus* by alkali treatment and alcohol precipitation, (provided by Marine Colloids, Inc., Rockland, ME) (Lot β282400) was administered in a 1% solution for 10 weeks as the sole drinking water source to six rhesus monkeys (three males and three females), weight 3–3.5 kg. Four rhesus monkeys (two males and two females) received drinking water alone and served as controls. Two animals from each group were injected iv with 10 mg/kg horseradish peroxidase type III (Sigma) dissolved in 0.5 ml saline 30 min prior to autopsy. All animals were killed by an iv overdose of Lethal Solution (Eli Lily), the entire colon excised and flushed with saline. For electron microscopy, 1-mm strips of flushed colon were cut in ice-cold 2.5% glutaraldehyde in Sorenson's phosphate buffer (pH 7.4); post-fixed in 1% phosphate buffered osmium tetroxide, dehydrated, and embedded in epon. For electron cytochemistry, strips of flushed colon were placed in ice-cold formol calcium and cut at 40 μm on a freezing microtome. Acid phosphatase activity was demonstrated by the method of Holt and Hicks (8). Peroxidase staining was demonstrated by the method of Graham and Karnovsky (9). Thin sections of colon were cut on a Sorvall MT 2B ultra microtome, and examined in a RCA-EMU 4B electron microscope. Colonic tissue was also fixed in ice cold absolute alcohol and stained with toluidine blue for the demonstration of metachromasia (10).

Results and discussion. Light and electron microscopy. Colonic submucosal macrophages from HMR-treated or control monkeys did not show any metachromasia with toluidine blue and were not observed to contain any fibrillar material. Occasionally, a macrophage was observed with a pigment-like inclusion, but no difference in vacuolation or pigment accumulation was observed between control and HMR-treated monkeys.

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Submucosal macrophages from the colon of rhesus monkeys administered C16 in drinking water were observed to contain lysosomes with fibrillar material which was metachromatic with toluidine blue (carrageeenan). This carrageenan was present in colonic submucosal macrophages even after a period of prolonged recovery (Fig. 1).

Electron cytochemistry. Colonic submucosal macrophages of either the control or HMR-treated monkeys were observed to have few, densely stained, primary lysosomes and no cytoplasmic acid phosphatase (Fig. 2). Primary endocytic vesicles containing HRP were observed fusing with primary lysosomes (Fig. 3). No macrophages were observed in association with polymorphonuclear leukocytes, nor were there any necrotic macrophages. Numerous colonic submucosal macrophages, from monkeys receiving iota carrageenan (C16), displayed many large, unstained, carrageenan filled lysosomes and cytoplasmic acid phosphatase (Fig. 4). Some of these colonic macrophages were observed to be necrotic with plasmallemmal breaks and were in close association with polymorphonuclear leukocytes (Fig. 5).

Electron cytochemical preparations for demonstration of exogenous peroxidase (HRP) showed that the primary endocytic vesicles containing the peroxidase reaction product failed to fuse with the large carragennan filled lysosomes in colonic submucosal macrophages from monkeys given iota carrageenan (Fig. 6).

Thus, a degraded iota carrageenan has been shown to be taken up and stored in colonic submucosal macrophages and this storage in some manner alters the functional state of these lysosomes. Recent papers (2, 6) have indicated that a pathological change in the gut in response to carrageenan administration (ulceration, squamous cell metaplasia, storage of iota carrageenan in lysosomes of macrophages of the lamina propria) was preceeded by lysosomal changes. Abraham *et al.* (2), have postulated that ulceration in the guinea pig is preceeded by release or labilization of carrageenan filled lysosomes from lamina propria macrophages with concomitant release of the acid hydrolases. Thus, the ulceration proceeds from the tissue to the lumen. However, ulceration in the monkey colon appears to proceed by a different mechanism. Benitz *et al.* (3) have

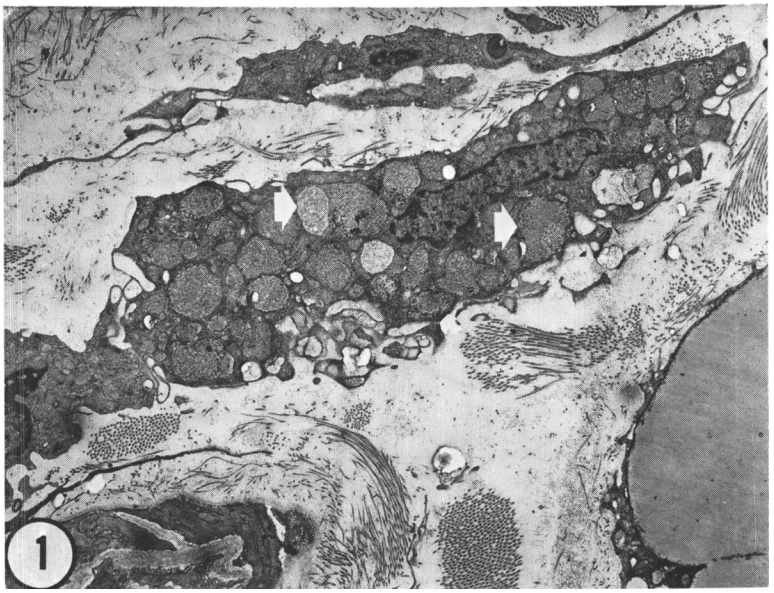


FIG. 1. An electron micrograph of a colonic submucosal macrophage from a rhesus monkey which received 2% C16 for 10 weeks and was allowed to recover for 24 weeks. The large vacuoles (arrow) are filled with an amorphous material (carrageenan). × 8000.

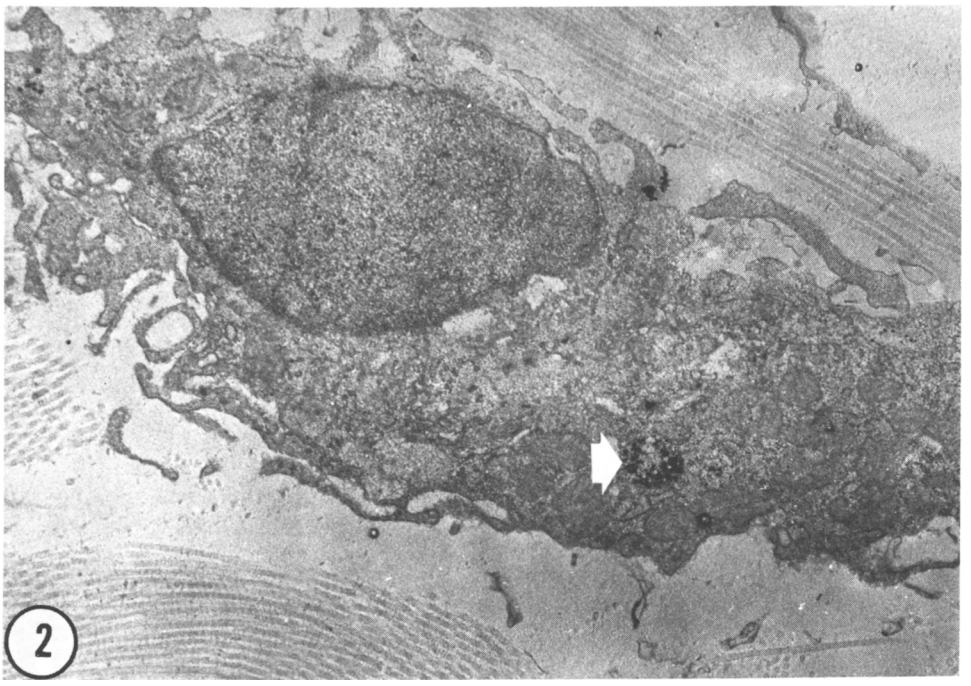


FIG. 2. An electron micrograph of a colonic submucosal macrophage, (stained for acid phosphatase) from a control rhesus monkey, showing small primary lysosome (arrow) and a lack of cytoplasmic acid phosphatase. × 15,000.

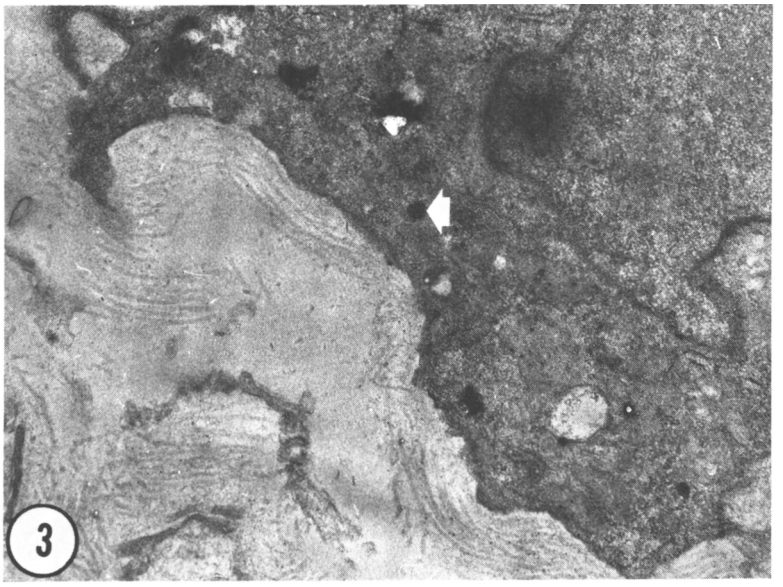
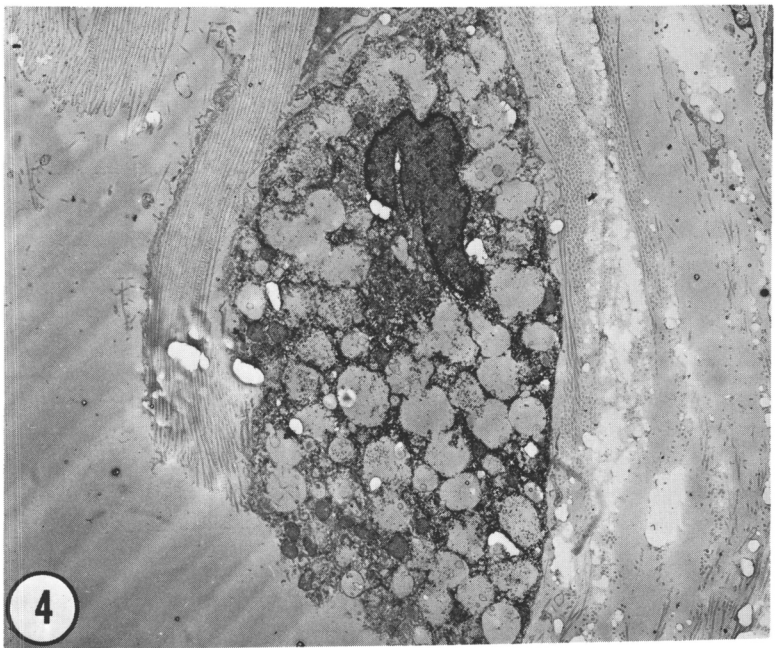


FIG. 3. An electron micrograph of a portion of a colonic submucosal macrophage, (stained for exogenous peroxidase) from a HMR-treated monkey. Numerous small endocytic vesicles are present (arrow). × 15,000.

described the colonic lamina propria macrophages from iota carrageenan treated rhesus monkeys as "normal in appearance and displayed no morphological characteristics suggestive of sulfated polysaccharide uptake; the absence of metachromasia was especially noteworthy," although ulceration of the colon had occurred. At present, no



FIGS. 4 and 5. Are electron micrographs of colonic submucosal macrophages, stained for acid phosphatase, from a rhesus monkey which received 2% C16 in drinking water for 10 weeks.

FIG. 4. Note the intense cytoplasmic acid phosphatase activity. × 8000.

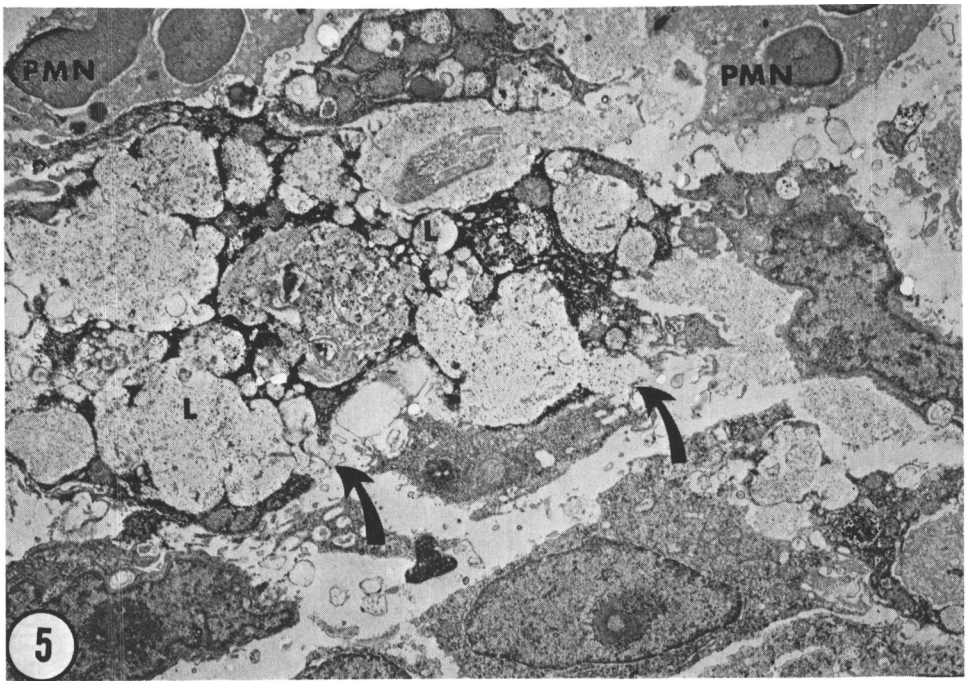


FIG. 5. Note the cytoplasmic acid phosphatase activity, plasmallemmal breaks (arrow), lysosomes with carrageenan (L), and close association to polymorphonuclear leukocyte (PMN). × 12,000.

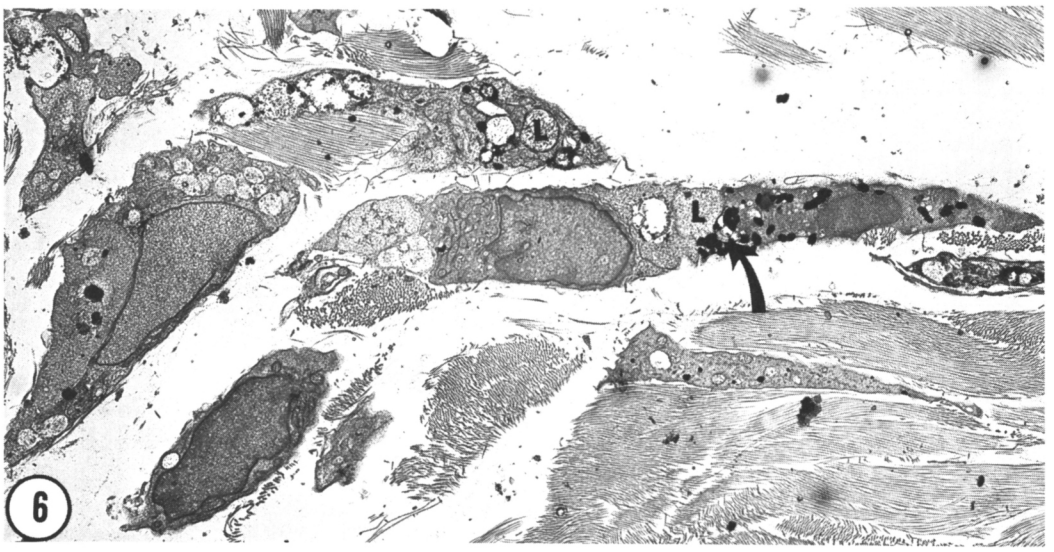


FIG. 6. An electron micrograph of colonic submucosal macrophages, stained for exogenous peroxidase (HRP) from a rhesus monkey which received 2% C16 in drinking water for 10 weeks. The endocytic vesicles with peroxidase reaction product have not fused with the carrageenan vacuoles (arrow) (L). × 5000.

pathological alterations have been correlated with the submucosal storage of iota carrageenan. Indeed, submucosal storage of carrageenan was noted in cecal macrophages of rhesus monkeys given iota carrageenan where ulceration does not occur (2). Continuing research into the relationship of these submucosal macrophages to lamina propria macrophages will establish whether these submucosal cells represent a pool of carrageenan laden cells which are in close association with the lamina propria pool or represent a distinct biohazard with as yet undiscovered pathological implications

Summary. Administration of a degraded iota carrageenan in drinking water to rhesus monkeys resulted in storage of carrageenan in colonic submucosal macrophage lysosomes that persisted even after prolonged recovery. This storage was accompanied by alterations in lysosomal function (appearance of cytoplasmic acid phosphatase, failure of HRP laden endocytic vesicles to fuse with the carrageenan laden lysosomes). Macrophage necrosis and leukocytic infiltration were also observed. Administration of a native (HMR) carrageenan caused no alterations in colonic submucosal macrophages, nor was any storage of HMR observed.

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