considerable water-binding capacity. In human aortae the greater part of the mucopolysaccharide fraction is chondroitin sulfuric acid but other mucopolysaccharides including hyaluronic acid seem to be present(12). While the hydroxyproline content showed no definite change, there was an increase in the hexosamine/hydroxyproline ratio indicating an increase in the amount of amorphous ground substance in proportion to the collagen.

The mechanism of the epinephrine effect is not clear. Theories have been advanced of vascular injury as a result of the variations in blood pressure accompanying the epinephrine injections; of an anoxic injury via the vasa vasorum; and of a direct effect on the cellular metabolism. The accumulations of acid mucopolysaccharides noted in these experiments is probably a reflection of reparative processes started by the epinephrine induced injury. Healing wounds contain large amounts of acid mucopolysaccharides, and the increased uptake of ^{35}S sulfate accord with previous observations of wound healing (13,14).

Summary. Alterations in rabbit aortae were brought about by intravenous administration of epinephrine. Microscopic examination revealed accumulation of metachromatic ground substance. The aortic content of hexosamine and water together with the uptake of ^{35}S sulfate was increased, whereas the amount of hydroxyproline was unaltered. The increase in the mucopolysaccharide/collagen ratio may indicate a reparative process.

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Local Morphological Response Following a Single Subcutaneous Injection of Carrageenin in the Rat. (25278)

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Various polysaccharides, mostly of bacterial origin, can induce inflammatory conditions in experimental animals(1,2,3,4). Subcutaneous injections of carrageenin, a polygalactose-sulfate of plant origin, have been used in different species for biochemical analysis of collagen formation(5,6,7,8). The present study of morphological response to a single subcutaneous injection of carrageenin in the rat was carried out to determine whether this plant material could be used as a stimulus to induce an inflammatory lesion.

Material and methods. Seven groups of 6 female Sherman rats (body-weight range 140 ± 10 g) were housed individually and fed Purina Laboratory Chow and tap water *ad lib.* All animals received a single subcutaneous injection of 0.5 ml of a 2% aqueous solution of carrageenin† (molecular weight 100,000 to 500,000 g/mol), on mid-line of back, halfway

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† Obtained through courtesy of Mr. Leonard Stolloff, Seaplant Corp., New Bedford, Mass.

between tail root and thorax. Groups of animals were sacrificed by chloroform after 1st, 2nd, 4th, 7th, 14th, 21st, and 28th day; and grossly recognizable abnormal structures of subcutaneous tissues were dissected and weighed on a Roller-Smith torsion balance. Dissected specimens were fixed in formalin-alcohol (1:9 V/V), and paraffin sections were stained with Masson's trichrome stain, the Alcian blue method for acid mucopolysaccharides, and with periodic-acid-Schiff reagent (PAS). Selected sections of each group were stained with Toluidine blue for metachromasia, and with Gomori's technic for reticulin fibers. In an additional weight study 5 groups of 12 animals were injected and sacrificed after 1st, 2nd, 4th, 7th, and 14th day. Grossly recognizable structures were removed, weighed on a Roller-Smith torsion balance, placed in tared vials, and dried to constant weight at 105 $\pm$ 5°C. Dry weights of residues were determined, and weights of volatile matter were obtained by difference.

Results. Immediately after administration of carrageenin, localized swelling of approximately 15 mm in diameter at site of injection could be noted which disappeared within an hour. All animals showed normal behavior, and gained weight. Gross and microscopic findings are described after various time intervals.

First Day—Gross findings. After removal of skin a slightly yellowish-white layer of very soft gelatinous consistency, poorly defined, was found in area from axillary to inguinal region. There was a slight hyperemia of surrounding tissues. *Microscopy.* Large areas of exudate with metachromatic staining properties and a rather weak positive reaction after Alcian blue stain indicated presence of carrageenin. Numerous ruptured collagen fibers which stained faintly blue with the trichrome method were found within this exudate, together with a large number of polynuclear and mononuclear leukocytes, most of them in diffuse distribution, but focal accumulation also occurred rather frequently. Erythrocytes were less commonly found, although in some instances parts of exudate were hemorrhagic. Occasionally, macrophages showed phagocytosis of carrageenin as shown by intensive intracellular metachromasia and positive, blue-green Alcian blue stain. Precipitation of fibrin was a rather common finding.

Second Day—Gross findings. Appearance of layer was almost identical with the findings after the 1st day with the exception that the lesion was less gelatinous. *Microscopy.* The findings were very similar to those described after the 1st day with the exception that the number of carrageenin-containing macrophages had increased and the uptake of polysaccharide material was more marked.

Fourth Day—Gross findings. A slightly yellowish-white layer of compact but still soft consistency was spread over a considerable area of the flank, and was poorly defined. There was a slight hyperemia of surrounding tissues. *Microscopy.* The large amount of stainable exudate had all but disappeared; only remnants were found. Their staining properties were essentially the same as described after the 1st day, indicating that carrageenin was still present. The major portion of carrageenin was found in numerous macrophages of foam-cell-like appearance after trichrome stain. Plasma of these cells showed a marked positive periodic-acid-Schiff reaction, strong metachromasia and a blue color after staining with Alcian blue. Numerous fibroblasts were intimately mixed with these macrophages. Multiple focal accumulations of leukocytes were found which often contained central necrotic areas consisting of cellular debris, fibrin, and ruptured collagen fibers.

Seventh Day—Gross findings. A thin whitish-pink layer of rather dense fibrous-like tissue was spread over a considerable area of the flank and was poorly delineated. No vascular reaction of surrounding tissues was visible. Pre-existing connective tissue could not easily be distinguished from the induced structures. *Microscopy.* Numerous fibroblasts, young capillaries and carrageenin-containing macrophages were found, forming granulation tissue which had walled off one or multiple necrotic areas. These necroses contained cellular debris, fibrin, and amorphous protein. Most macrophages at this time maintained their foam-cell-like appearance after trichrome stain and Alcian blue method, but after the periodic-acid-Schiff method and the

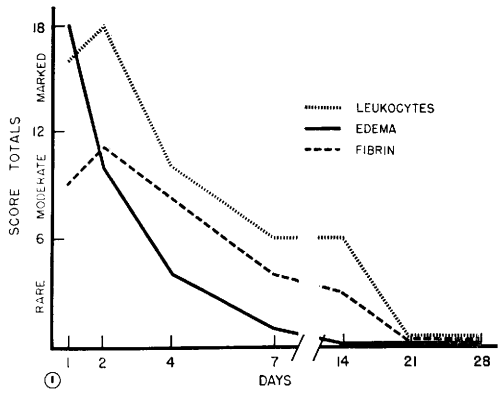
Toluidine blue stain the phagocytized polysaccharide appeared as multiple small droplets. Curly strands of collagen fibers and a fine network of reticulin fibers were found between the cellular elements. Some apparently newly-formed fat cells contained very small amounts of polysaccharide material which could be seen as little globules in a barely stainable cytoplasm. These were attached to certain parts of the cell border. The rest of the cell was occupied by a large optically empty area, which indicated former presence of fat.

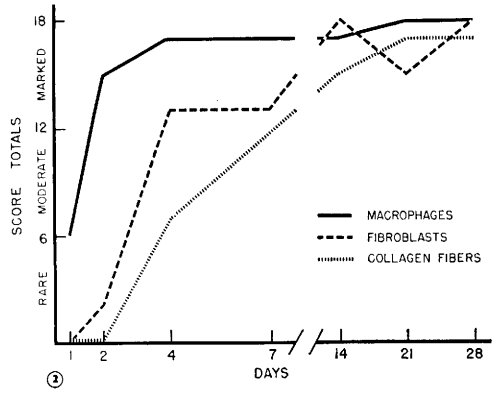
Fourteenth Day—Gross findings and microscopy were similar to observations after 7th day.

Twenty-first Day—Gross findings. There was a thin whitish-gray layer of compact fibrous tissue spread over a smaller area on the flank. The structure was not easily distinguished from surrounding tissues. *Microscopy.* Almost no necroses were found at this stage. In several areas of previously described granulation tissue, a decrease of number of macrophages, fibroblasts, and capillaries was found together with a marked fibrosis, *i.e.*, new formation of collagenous and argyrophilic fibers. Other parts of the granuloma still showed numerous macrophages containing polysaccharide material (positive reaction with PAS, Toluidine blue, and Alcian blue) and fibroblasts which were intimately mixed and packed in a firm meshwork of reticulin fibers.

Twenty-eighth Day—Gross findings and microscopy were similar to observations after the 21st day. An attempt was made to obtain a crude quantitative estimate of different characteristic tissue components of the removed structures after different time intervals.

All slides were examined and the following changes were graded: edema, polynuclear leukocytes, fibrin, macrophages, fibroblasts, and formation of collagen fibers. Grading was done by assigning 0 for absent, 1 for rare, 2 for moderate, and 3 for marked occurrence. Since 6 specimens of 6 different animals were used, the evaluation was based upon the following score totals: 0 to 6 = rare; 6 to 12 = moderate; and 12 to 18 = marked. The





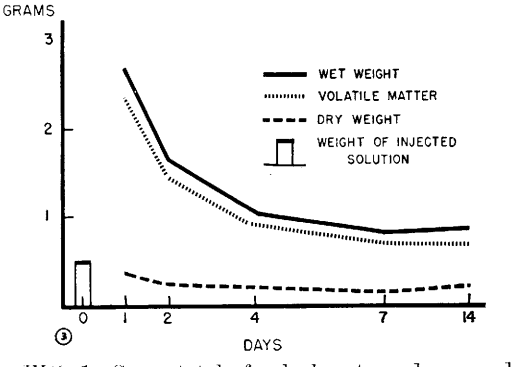


FIG. 1. Score totals for leukocytes, edema, and fibrin of carrageenin-induced lesions, 6 rats/point, at different time intervals after inj.

FIG. 2. Score totals for macrophages, fibroblasts, and collagen fibers of carrageenin-induced lesions, 6 rats/point, at different time intervals after inj.

FIG. 3. Weight relations of carrageenin-induced lesions at different time intervals, 12 rats/point.

results are shown in Figs. 1 and 2.

These morphological studies show that a single subcutaneous injection of 0.5 ml of a 2% aqueous solution of carrageenin in rats caused a localized response which could be

divided easily into 2 stages. The initial phase (1st to 4th day) was characterized by one or more central necrotic foci, surrounded by exudate with leukocytic infiltration, precipitated fibrin, slight hemorrhage and a moderate proliferation of macrophages containing carrageenin. These findings were interpreted as an acute localized inflammatory response in the form of an abscess.

After 4th day, the abscess was walled off; debris and exudate gradually disappeared and were replaced by proliferating macrophages and fibroblasts and by formation of new fibers and capillaries. Thus, the abscess was transformed into a granuloma in the process of repair with ultimate scar formation.

To quantitate the amount of initial inflammatory exudate, *i.e.*, fluid, an additional weight study was carried out as described under Material and methods. Relationships between wet weight, dry weight, and weight of volatile matter of the excised lesions at various intervals up to 2 weeks are shown in Fig. 3.

Discussion. So far only Williams(9) has furnished information about the morphological findings at different time intervals after a single subcutaneous injection of carrageenin in the guinea pig. His observations, however, differ somewhat from our findings in the rat, since he did not find fibrin or fibrinoid material, and only a moderate polymorphonuclear leukocytic response in early stages of the induced lesions. Our observations in the rat indicate a marked leukocytic response and a moderate precipitation of fibrin in the lesion as a common finding during the 1st to 4th day. Species differences may also account for the difference in maximum wet weight of removable srtuctures. In the guinea pig, Robertson and Schwartz(5) reported a maximum at 14th day after a single subcutaneous injection of 16.6 mg of carrageenin/100 g body weight, and Jackson(7) found a maximum at the 7th day after 7.7 mg/100 g body weight. In the rat we observed a maximum wet weight after 24 hours using 7.2 mg of carrageenin/100 g body weight.

All these differences indicate that carrageenin stimulates a more violent acute inflammatory response in the rat, whereas the guinea pig reacts in a more chronic way by forming excessive granulomatous structures.

R. Meier *et al.*(3) used another polygalactoside, namely 5 mg of purified agar/105 g body weight in rats, and observed only a very weak reaction by the wet weight (30 mg) of lesions removed after 7 days. Although we have used larger animals (140 g) and a larger amount of polygalactoside (7.2 mg), our mean wet weight after 7 days was still 920 mg, indicating a more violent and extensive response.

Whether this marked difference is due to high degree of sulfation, approximately 23% of carrageenin in comparison with 1.3 to 1.4% of agar(10) remains to be elucidated.

These studies show that carrageenin, administered as a single subcutaneous injection in the rat, is an irritant which produces a local response. This reaction, a well-defined accumulation of exudate containing fibrin, polynuclear leukocytes, and erythrocytes is, by definition of Boyd(11), inflammation.

Summary. 1. A single subcutaneous injection of carrageenin in rats caused a local tissue response, which from the 1st through the 4th day could be defined as an inflammatory lesion in the form of an acute abscess, which later (5th through the 28th day) was gradually transformed into a polysaccharide-containing granuloma. 2. Weight data indicate a maximum exudative response after 24 hours.

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