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INVERTEBRATE GLYCOPROTEIN-INDUCED INFLAMMATION IN RATS

Deborah I. Asrican Bailey

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

at

The University of the Pacific

1977

This thesis, written and submitted by

Deborah I. Asrican Bailey

is approved for recommendation to the Committee on Graduate Studies, University of the Pacific.

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Dated August 1, 1977

DEDICATION

I dedicate this thesis to the memory of Ed and Doris Bailey.

> As long as one is remembered, he will never truly die. "Dad" and "Mom," you will therefore always live because you will always be in the hearts of the ones who loved you.

ACKNOWLEDGEMENTS

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INTRODUCTION

Inflammation is a normal protective response of the body to a noxious stimulus. It is a response aimed towards localizing and neutralizing the effects of that noxious stimulus and towards effecting tissue repair in those areas damaged by that stimulus. Since it is a process directed toward the maintainence of the constancy of the body, inflammation may be considered a homeostatic mechanism. However, like other homeostatic mechanisms, the inflammatory process is subject to loss of control and sometimes can cause more harm than the original injury.

When tissue injury is caused by a single finite event, such as mechanical trauma, a thermal or chemical burn, or a single exposure to a non-replicating antigen, the inflammatory process is "acute" and progresses smoothly from injury to healing. Under these circunstances, the process is truly beneficial and provides an example of a homeostatic mechanism restoring the affected tissue to its former normal and helathy state. In contrast, when the injurious agent is a self-replicating parasite such as a bacterium or virus (which cannot be destroyed or readily eliminated), the ensuing inflammatory response becomes more complex and does not always progress smoothly from injury to healing. Under

these circumstances, the inflammatory response becomes "chronic." Homeostasis is not achieved; instead, tissue injury persists, not only as a result of the continuing existence of the noxious agent, but also as a result of the "loss of control" which develops within the inflammatory response itself.

The expression of inflammation, therefore, represents a spectrum, with the response varying from an acute, transient, and highly localized response to a complex, chronic, and sustained response, perhaps involving the whole organism. In spite of the fact that many types of stimuli can cause inflammation and that many degrees of inflammation can result, the inflammatory process itself always seems to follow an orderly biochemical and morphological sequence, and the difference in complexity between the types of inflammatory states is more quantitative than qualitative (1).

The clinical expression of the process was described as early as the first century A.D. by the Roman encylopedist, Celsus. His classic description of the process consisted of calor (heat), rubor (redness), tumor (swelling), and dolor (pain). Galen (A.D. 130-200) subsequently added function laesa (loss of function) (2). We now know that each of these observable manifestations of inflammation represents certain biochemical and morphological changes.

The pattern of biochemical and morphological changes which occur following any type of tissue injury may be summarized as follows:

(i) vascular changes in the microcirculation,

(ii) migration of cellular mediators to the injured area and a "walling off" process, plus

(iii) connective tissue proliferation.

Vascular Changes. Immediately following tissue injury or irritation, there is a brief arteriolar vasoconstriction (3), followed in a few seconds by vasodilation of the arterioles, capillaries, and venules (4). During this period of vasodilation, blood flow first quickens, then becomes sluggish or even stops. This stasis of blood is due to a loss of water primarily from the venules. This water loss is due to marked increase in the permeability of the venules to the plasma proteins which, upon leaking into the extravascular spaces, disturb the osmotic balance which previously maintained water within the vascular system. The endothelial cells lining these vessels may also become damaged and swollen an narrow the lumen of the microcirculatory system. In addition, polymorphonuclear phagocytes (PMN phagocytes) adhere and begin to accumulate along the margin of the vasculature and further narrow the lumen. All this results in further stasis of blood and increased edema formation (5).

Many possible mediators have been implicated in this "initial vascular" phase of inflammation. Histamine, bradykinin, and the prostaglandins are a few examples of the chemical substances that are found at the inflammatory site and probably responsible for the vasodilation and increased vessel permeability (1).

As soon as tissue injury occurs, the mast cells release both histamine and heparin. Histamine causes an immediate increase in vessel permeability. The role of heparin is more difficult to evaluate, but it has been suggested that it serves to prevent thrombosis of the small vessels at the site of the injury. Slightly later, kinins are produced and these potent substances have the capacity to activate pain sensors in addition to a histamine-like effect on venule permeability (6). The kinins are formed from an

ever-present plasma kininogen, and their formation is generated via a sequential involvement of three factors: Hageman factor, permeability factor, and kallikrein (7). Both histamine and the kinins have been shown to increase venule permeability by causing gaps to appear between the endothelial cells, thus allowing interendothelial leakage of protein and exit routes for phagocytes (8).

The prostaglandins may also be responsible for some of the vasodilation witnessed during this early phase of the inflammatory response. Prostaglandins have been detected in blister fluid and perfusates of the skin of patients with contact dermatitis. In animals, they have been detected in experimental inflammatory states such as skin burns in dogs and carrageenin-induced pedal edema in the rat (9). While there is no doubt that prostaglandins are formed in inflammatory states, it should be understood that not all species of prostaglandins promote vasodilation. One prostaglandin may actually have an opposing action to that of another prostaglandin in an inflammatory state (10). Therefore, one has to be concerned both with the identification of the prostaglandic inflammogens present and the ratio between the pro-prostaglandic inflammogens and the anti-prostaglandic inflammogens which originate within the injured tissue.

In summary, the initial vascular phase of inflammation appears to be initiated by histamine released from the mast cells and prolonged (although only briefly) by polypeptide kinins and the acid prostaglandins. Since these tissue hormones or autacoids are formed or released <u>in situ</u> and their duration of action is so brief, it is hard to believe that they could be "primarily" responsible for the tissue injury of chronic inflammation. Nevertheless, they are

undoubtedly very important in the triggering of the acute inflammatory state.

Serotonin, the catecholamines, and the adenylates (cyclic AMP and ADP) may also be classified as mediators in the inflammatory response, although their roles are much less clearly defined. For example, the effect of serotonin shows variability between species and often little resemblance to changes seen in man. In rodents, serotonin causes a combination of arteriolar dilation and separation of endothelial cells. Yet in man, although it has been found in inflammatory exudates, it has no prominent effects upon vasodilation (3). The permeability-enhancing property of serotonin in man thus seems nonexistent, but this does not eliminate serotonin from having a possible role as a mediator in inflammation since there is ample evidence that mediators are chemically, physiologically, and pharmacologically interrelated (11). Thus, the active contribution of serotonin may not be dramatic, but it may be critical for the direct effect of other mediators.

The catecholamines generally are not considered as mediators of inflammation. Yet, like serotonin, they too may alter the manifestations of the inflammatory process. It is thought that they may act locally during the inflammatory process as endogenous anti-inflammatory hormones (12). More information, however, is needed before the direct and/or indirect roles of the catecholamines during inflammation can be defined. The role of the adenylates is much like that of the prostaglandins. They may be key participants in amplifying the inflammatory state, but again, since it has been demonstrated in other contexts that cyclic GMP may elicit responses diametrically opposed to those elicited by cyclic AMP, it is their ratios which must be defined and not merely their presence (10).

Migration of Cellular Mediators to the Injured Area. Immediately following the early vascular events, a greater number of PMN leukocytes appear at the injured area. Several effects occur to cause the movement of these cells (particularly neutrophils) to the injured area. First, the neutrophils stick to the walls of the damaged capillaries. This is a process known as margination. The total number of granulocytes available in the blood during tissue injury is increased above normal, since a globulin substance known as leukocytosis-promoting factor is liberated by inflammed tissues. This factor diffuses into the blood and bone marrow where it has the effect of causing large numbers of granulocytes, expecially neutrophils, to be released from the storage areas in the bone marrow into the blood (13). The second effect is a chemotaxis of the neutrophils toward the damaged area. This is caused by certain of the substances released or activated by the inflammatory stimulus. Many of the suggested inflammatory mediators as well as the immunologically activated complement components are purported to have chemotaxic properties (14). Electron microscopic studies have demonstrated that the PMN phagocytes leave the inflammed vessels by inserting pseudopodia down through the interendothelial gaps, with cytoplasm then flowing through these pseudopodia until the cell has completely left the lumen. It then lies between the endothelium and the endothelial basement membrane. The PMN phagocyte then passes through the basement membrane, perhaps by enzyme action, with the membrane reforming immediately after its passage (15). Once these phagocytes have emigrated through the vessel wall, they begin to ingest and digest or kill the "noxious"

agent. This is accomplished by the release of lysozomal enzymes which unfortunately may also cause further tissue destruction and edema formation. Thus, it is apparent that leukocytes play more than one role in the inflammatory process. On the one hand, they seem to help resolve inflammation by ridding the area of the "noxious stimulus," while on the other hand, they serve to promote the process by causing further tissue damage and consequently the release of more inflammation-promoting substances (16).

PMN leukocytes are the predominant cells in the inflammed tissue during the early phases of the inflammatory process. Later, however, monocytes and macrophages are more numerous. There are several factors responsible for this shift. First, it appears that mononuclear phagocytes migrate slower than PMN phagocytes. Second, the mononuclear phagocytes depend, in part, upon the polymorphs for their chemotactic signal. Third, and perhaps most important, however, is the fact that with time the affected area becomes acidic, and PMN cells, particularly neutrophils, cannot live in an acid environment, whereas macrophages live well under acid conditions -- their proteolytic enzymes actually becoming more active. Fourth, is the fact that mononuclear phagocytes are longer lived than the PMN phagocytes and are capable of cell division at the site of inflammation (5).

Once the vascular and cellular events are activated, the process of "walling off" the inflammed area is initiated. The early isolation of the area probably is advantageous for it allows time for the phagocytic cells to assemble in the inflammed area. This "walling off" process primarily is due to the formation of

loosely organized fibrin clots which obstruct the microvessel flow. This obstruction, accompanied by the previously described vascular stasis, is responsible for one of the classic symptoms of inflammation -- edema (1).

The inflammatory process, as described up to this point, has been termed the "exudative degenerative" stage of inflammation. As this phase is terminated (by processes that are not well understood), the final stage of inflammation characterized by fibrous tissue growth is begun (1).

Connective Tissue Proliferation. The primary stimulus which initiates new connective tissue synthesis at the inflammatory site is not yet known. It is known, however, that the repair process begins as a proliferation of fibroblasts and multiplication of small blood vessels by mitosis of connective tissue and endothelial cells. As the cellular proliferation penetrates the exudate, it produces a highly vascularized, reddish mass termed granulation tissue. Eventually, this granulation tissue completely replaces the exudate. The amount of fibrous tissue growth is dependent on the type of inflammation. Both acute and chronic inflammatory injuries follow similar patterns of tissue repair, but quantitatively larger amounts of fibrous tissue are formed in the course of tissue healing following chronic inflammatory processes (1). This stage of the inflammatory process, like the other stages that have been discussed previously, is subject to loss of homeostatic control. The formation of excessive fibrous tissue can lead to severe complications, and such a loss of control is one of the symptoms of rheumatoid arthritis (1).

Research Rationale. The preceding description of the inflammatory process is brief and simplistic, but it is important because it points out that there are many potential ways whereby a pharmacologic agent might exert its effects in the treatment of inflammatory disorders. For example, a pharmacologic agent could modify the inflammatory process by (i) affecting the etiological factors (i.e., the primary causes); (ii) affecting the mediators of the initial tissue injury which are released by, or produced in response to, the etiological factors, and (iii) affecting the normal and/or abnormal processes which are evoked by the tissue injury in an attempt to restore function. While inflammation per se has been defined sufficiently to permit a rational approach to the search for drugs to modify its processes, satisfactory animal models for studying inflammation are not available. For example, the widely used carrageenin-induced pedal edema system in rats used to induce acute inflammation for drug screening of "anti-inflammatory" drugs often turns up false positives (17). Carrageenin is an agar-like carbohydrate polymer from the Irish Moss plant: Chondrus crispus (Linne) Stackhouse (Gigartinaceae). Carrageenin is considered to be a sulfuric acid ester of a complex galactose polysaccharide (18).

The present program of research, therefore, was designed to attempt to gain new insights into the possible etiology of inflammatory states -- for when new information concerning the etiology of these disease states becomes available, more meaningful screening procedures for anti-inflammatory drugs will certainly be devised. Two preliminary studies have been published (19, 20).

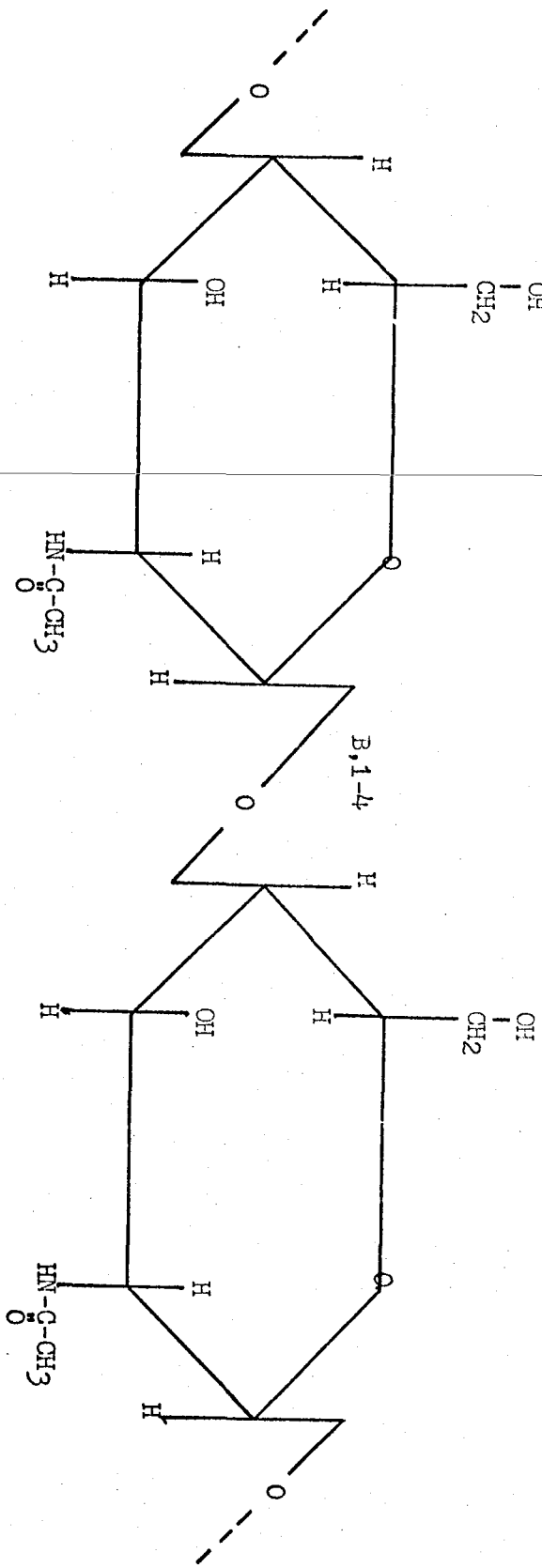
Chitin has been increasingly identified in the cell walls

of fungi, yeasts, and certain microorganisms (21). Therefore, one might predict that any bacterial or fungal invasion could leave behind undegraded chitin or partially-degraded chitin as a permanent resident in the host tissue. While the immune response, phagocytosis, enzymatic catabolism or other defense mechanisms could destroy the causative organism, chemically resistant chitin could be left behind. This residue, being foreign to the body, could initiate and perpetuate the inflammatory response. Since a chitinous cell wall is so extremely thin and its refractive index so near to that of water, these residues would be effectively invisible to most detection methods (22). No living organism has ever been found in the lesions of rheumatoid arthritis.

Chitin also occurs rather widely throughout the animal kingdom, especially in the connective tissue of invertebrates (23). Its chemical nature has been studied extensively, and it has been identified as a polymer of N-acetylglucosamine -- more specifically, the carbohydrate moeity has been identified as the B, 1-4 linked polymer of N-acetylglucosamine (24, 25). See Figure A. Futhermore, it has been noted that the chitin seems to be aggregated to various proteins in the connective tissue of the invertebrates -- the individual proteins being dependent upon the particular species of invertebrate (23). The protein which is connected to the chitin is important since it seems to protect it from chitinase and chitobiase (26).

With the above in mind, this study was directed towards determining whether or not various types of chitinous materials were indeed capable of inducing an inflammatory response upon injection. Secondarily, by the use of known anti-inflammatory drugs, this study was also directed toward determining the possible

Fig. A: Structure of Chitin


O
H
H
OH
H
OH
CH2
HN-C-CH3
O
H
O
β,1-4
O
H
H
OH
H
OH
CH2
HN-C-CH3
O
H
O

mechanism(s) of action by which these chitinous materials could induce inflammation.

The various chitinous materials studied include the following: (i) float material of Velella lata (a pigmented jelly fish commonly known as the purple sailor and frequently found washed up on California beaches), (ii) gladius or pen material of Loligo opalescens (a common squid found in Pacific coastal waters), and finally (iii) the shell material of the common shrimp. Polyglucose (from cotton) was also investigated. Carrageenin was used as a reference control.

The ability of invertebrate connective tissue to cause an inflammatory response in mammals raises intriging chemical questions. The answers to these questions are hidden in the organization of the interacting moieties. For this reason, deproteinated and partly deacetylated forms of the above mentioned chitinous materials were also studied for their respective phlogistic potentials.

Since the natural forms consist of the chitin carbohydrate moiety (poly N-acetylglucosamine) and its corresponding protein, these natural forms may be considered true glycoproteins. The deproteinated forms consist only of the carbohydrate moiety, poly N- acetylglucosamine, while the partly deacetylated forms largely consist of glucosamine (27).

Investigation into the potential phlogistic capacity of these forms should allow one to narrow the inflammatory moeity down until the minimum molecule(s) necessary for inducing the inflammatory response has (have) been isolated. i.e., one should be able to define the type of inflammation induced by the natural chitin-based glycoproteins and to determine whether or not it is the participation of the glycoprotein complex, the carbohydrate

moiety alone, or of the protein alone which is responsible for the induction of the inflammatory response.

This research should add to our knowledge about the etiology of the inflammatory diseases of connective tissue. To date, the failure to detect a causative organism or chemical activator for such diseases has severely hindered effeorts to either prevent the initiation of such disease states or to effectively alleviate the clinical effects that these disease states produce. If the exact chemical species triggering the inflammation could be determined, this could be significant in opening new avenues for drug treatment and design.

MATERIALS AND METHODS

Rat pedal edema assays:

Male Sprague-Dawley rats[a] weighing between 125 and 150 grams were used. Upon arrival at our facilities, the animals were randomized, marked, and assigned to experimental groups; each group consisting of from seven to ten animals. Animals were housed in pairs in cages with the following dimensions: 24.1 x 17.8 x 17.8 cm. The cages were made of galvinized sheet metal sides and wire-mesh floors. The environmental temperature was maintained at 72-75°C and the quarters were illuminated from 7 a.m. to 5 p.m. daily to simulate diurnal patterns. Block-type lab chow[b] (placed on the cage floors) and tap water (bottles attached to the cages) were supplied ad libitum. All animals were housed under these conditions for at least one week prior to testing.

At 0 hr., the animals were weighed, injected with sodium pentobarbital[c] (30 mg./5 ml./kg. intraperitoneally) and then given a subplantar injection of one of the test phlogistic agents. This injection

---

a - Horton Laboratories Inc., Oakland, California.
b - Purina Laboratory Chow. Crude protein not less than 23.0%; crude fat not less than 4.5%; crude fiber not more than 6.0%; and ash not more than 9.0%
c - Robinson Laboratories Inc., San Francisco, California; sodium pentobarbital U.S.P., Catalogue number 0140-01, control #SC8403.

was made into the animal's right hind paw using a 5/8-inch, 25-gauge needle,[a] and a micrometer syringe.[b] The legs of each animal were marked at the ankle joint using a permanent-type felt-tipped marker and all paw volumes were recorded plethysmographically to this point at -5 min., +5 min., +1 hr., +3 hr., +5 hr., and daily thereafter through +20 days.

The apparatus used for recording paw volumes consisted of a cylindrical plastic reservoir (approximately 25 ml. in volume) filled with mercury and connected by an S-shaped glass tube (4 mm. I.D.) to a Statham Physiological Pressure Transducer,[c] Model P23BB (Serial #14799) filled with distilled water. Volume changes in the mercury reservoir due to the presence of an immersed object could then be measured as pressure changes. These changes were modified by the transducer into electric signals which were then amplified and recorded on heat-sensitive graph paper by a Beckman Dynograph (Type RS)[d] equipped with a hot-wire stylus (see addendum A).

Calibration of volume changes was accomplished by immersing a cylindrical Perspex rod attached to a stereotaxic unit (Narishiga #2321) into the mercury reservoir. Each calibration mark on the rod was calibrated to displace 0.5 ml. of water, and the Dynograph was calibrated so that the recording stylus correspondingly would move 5 mm. on the recording paper for each mark. Plethysmographic determinations of rat paw volumes were not attempted until a linear displacement

---

a - Becton, Dickinson & Co., Rutherford, New Jersey
hyperchrome stainless hypodermic needle with regular point.
b - RGI Inc., Vineland, New Jersey.
c - Manufactured by Statham Laboratories, Inc., Hato Rey, Puerto Rico.
d - The Beckman Dynograph (type RS) used was manufactured by Beckman Instruments, Inc., Schiller Park, Illinois.

by successive immersions of the rod at varying depths into the mercury had been demonstrated (28).

The animals were held loosely while the injected and then the contralateral paw volumes were recorded. This could be accomplished by fully extending the legs by exerting pressure just above the knee. A total of three immersions were used to determine the average paw volume of each rat at each time period.

Degree of edema was calculated by subtracting the control paw volume (taken immediately before phlogiston injection) from the inflammed paw volume, and by computing the mean and standard error for each group. Statistically significant differences between groups were documented using Student's t test.

The following materials were studied for their phlogistic capacities: Mycobacterium butyricum,[a] carrageenin,[b] cotton,[c] and the whole, deacetylated, and deproteinated forms of the float material of Velella lata,[c] the gladius or pen material of Loligo opalescens,[c] and the shell material of the common shrimp.[c] All materials were ground to 200-mesh powders before being suspended in either light mineral oil[d] at a concentration of 5 mg./ml. and injected at a volume of 0.05 ml., or in 0.9% saline[e] at a concentration of 10 mg./ml. and injected at a volume of 0.10 ml.

Short term single-dose drug studies:

Male Sprague-Dawley rats[f] weighing between 125 and 150 grams

---

a - Difco Laboratories, Detroit, Michigan; Catalogue #0640-25.
b - Seaplant Chemical Corporation, New Bedford, Mass.; Seakem Type I Lot #312503.
c - Received from R.L. Gainey, Chief Chemist, FMC Corp., Modesto, Calif.
d - Light mineral oil N.F.
e - Fisher Scientific Co., Fair Lawn, New Jersey. Biological Grade, Lot #733400.
f - Horton Laboratories Inc., Oakland, California.

were maintained as in the rat pedal edema assays. At -1 hr., the animals were taken off food, weighed, and dosed orally with either a 0.25% agar solution or an anti-inflammatory drug suspended in a 0.25% agar vehicle, using an 18-guage oral feeding needle. At 0 hr., a 0.10-ml. subplantar injection of either carrageenin[a] or whole Velella float[b] material (suspended at 10 mg./ml. in 0.9% saline) was made into the right hind paw of the animals using a 5/8-inch, 25-gauge needle[c] and a 2-ml. micrometer syringe.[d] After phlogiston injection, food was again allowed ad libitum. Paw volume measurements were recorded at -5 min., +5 min., +1 hr., +3 hr., and +5 hr. All animals were injected with sodium pentobarbital[e] 30 mg./5 ml./kg. IP in order to insure precise subplantar pedal injections.

The following drugs were studied for their ability to inhibit inflammation induced by either carrageenin or whole velella float materials: aspirin,[f] hydrocortisone,[g] cryogenine,[h] phenylbutazone,[i] indomethacin,[j] and chlorpromazine.[k] All of these drugs were suspended by trituration in 0.25% agar,[l] and administered at a constant dosage volume of 10 ml./kg.

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a - Seaplant Chemical Corporation, New Bedford, Mass.; Seakem Type I, Lot #312503.
b - Received from R.L. Gainey, Chief Chemist, FMC Corp., Modesto, Calif.
c - Becton, Dickinson, & Co., Rutherford, New Jersey; hyperchrome stainless hypodermic needle with regular point.
d - RGI Inc., Vineland, New Jersey.
e - Robinson Laboratories Inc., San Francisco, California; Sodium pentobarbital powder, U.S.P. Catalog #0140-01, Control #SC8403.
f - Mallincdrodt Chemical Works, St. Louis Missouri.
g - Nutritional Biochemical Corporation, Hollywood, Calif.; Control #2912.
h - Compliments of William Watson who isolated the cryogenine from Heimia salicifolia, using the procedure of Stanley T. Omaye (29).
i - Ciba Pharmaceutical Co., Summit, New Jersey.
j - Merck, Sharp, & Dohme Research Lab, Rahway, New Jersey.
k - Smith, Kline, & French Laboratories, Philadelphia, Pennsylvania.
l - Difco Laboratories, Detroit, Michigan; Difco Bacto Agar, Lot #0140-01.

The amount of protection provided by each of the drug treatments against inflammation induced by either carrageenin or whole velella float material was determined by subtracting the control paw volume (i.e., the -5 min. reading which was taken prior to the injection of the phlogiston) from the inflammed paw volumes, and by computing the mean and standard error for each group. Statistically significant differences between treatments was determined by use of Student's t test.

Mycobacterium butyricum adjuvant preparation:

The methods used in this preparation procedure were essentially the same as those described by Kosersky (30). One hundred mg. of heat-killed desiccated Mycobacterium butyricum[a] was triturated in a glass mortar with light mineral oil N.F., to a final concentration of 5 mg./ml. This suspension was then more finely divided and uniformly suspended by using a tissue homogenizer and pestle[b] fitted to an electric motor[c] and spun at approximately 1500 R.P.M. The pestle was moved up and down about 10 times. The adjuvant was poured into 10-ml. parenteral vials, covered with rubber serum caps, and autoclaved at 120°F, 20 p.s.i. for 20 min. The adjuvant was then stored at 4°C for a week prior to use. Before injection into animals, the adjuvant was warmed to room temperature.

Chemical preparation of chitinous phlogistons:

Whole, deacetylated and deproteinated forms of the floats

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a - Difco Laboratories, Detroit, Michigan. Catalog number 0640-25.
b - Kontes Glass Co., Vineland, New Jersey. Kontes glass jacketed tissue homogenizer, mortar #71, pestle #52.
c - Talboys Engineering Corp., Emerson, New Jersey, model 106.

of Velella lata, of the gladius or pen material of Loligo opalescens, and of the shells of the common shrimp, were prepared by Ralph L. Gainey[a] in the following manner: Natural whole floats of the Northern California variety of Velella lata[b] were dried and freed of sand and extraneous materials. Gainey has documented that the floats are approximately 3.0 - 4.0% inorganic material, 0.2 - 0.3% lipid, 55.0 - 58.0% carbohydrate, and 42.0 - 45.0% protein by dry weight (31). The samples of natural whole float material that were received were labelled K-749000 and RG:740405A. Deacetylated Velella lata was prepared by allowing the dried, washed floats to set for 36 hrs. in 6N KOH followed by neutralization and washing. The samples of deacetylated velella that were received were labelled K-749005 and RG:740405B. Deproteinated Velella lata was prepared by allowing the dried washed floats to set for 8 days in 6N KOH at room temperature followed by subsequent neutralization, washing, and drying. The sample of deproteinated velella that was received was labelled RG:730621.

The whole gladius or pen material of Loligo opalescens was prepared by washing and drying the chitinous pens. The pens were then diced and ground in a Spex grinder for 2-3 min. to a fine powder. This powder was placed in a 250-ml. erlenmeyer flask with 100 ml. 6M KOH at room temperature (22°C) for 11 days. After 11 days the material was filtered and washed a number of times with deionized water, followed by a wash with 0.5 M HCl and several more washings with deionized water. Three washings of acetone

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a - R.L. Gainey, Chief Chemist, FMC Corp., Modesto, California.
b - Velella lata: Phylum: Coelenterata; Class: Hydrozoa; Order: Hydroida; Suborder: Chondrophors.

were used to promote drying and the material was placed in the oven for 10 min. at 110°C. The sample of whole loligo pen material which was received was labelled #139-2. Deacetylated loligo pen material was prepared by taking ordinary pen material and washing it in water upon removal from the animal. It was then dried in air at room temperature and ground in a Spex grinder to a fine powder. The powder was washed 3X with 0.5 N HCl (to remove residual iron) and then washed 3X with acetone (to aid drying). It was stored for less than four weeks in a glass vial at room temperature and then boiled in 3N KOH for 25 hrs. It was then washed 3X with 0.5 N HCl and finally 3X with acetone and placed in a glass vial which was labelled RG:740715 upon receipt. Deproteinated loligo pen material was prepared by placing cleaned dried whole pen material in a 25-ml. flask and covering it with 6 N KOH. After 9 days at room temperature, the pen material was filtered and washed with water until the filtrate no longer gave a pink color to a phenolphthalein solution. It was washed 1X with 0.5 N HCl and 2X with acetone. A final wash with water was followed by drying at 110°C for 10 minutes. The sample of deproteinated loligo pen material that was received was labelled #152-B.

Whole shrimp shell material was prepared by washing the shells of fresh shrimp to free them of adherent material, and by drying them at room temperature. The shells were ground in a Spex grinder for 3 minutes. The ground shells were transferred into a sintered glass filter and washed 2X with 0.5 N HCl, 2X with acetone, and dried at 110° for 20 minutes. The sample of whole shrimp that was received was labelled #152-A. Deacetylated shrimp shell material was prepared by allowing the dried washed whole shells

to set for 24 hrs. in 1N KOH at 110°C. The sample of deacetylated shrimp that was received was labelled RG:740225. Deproteinated shrimp shell material was prepared by washing and drying the shells of fresh shrimp (identical to sample #152-A). These shells were then placed in 6N KOH for ten days at room temperature after being washed with 0.5N HCl and acetone. The deproteinated shells were washed on a sintered glass filter until the filtrate failed to give color to a phenolphthalein solution, indicating base removal. After washing 3X with acetone, the shell material was dried for 10 min. at 110°C and placed on a Mettler LP 11 drying balance and brought to a constant weight. The sample of deproteinated material was labelled #152-C.

All treated or untreated chitinous materials discussed in this section were ground to 200-mesh powders before they were suspended in either agar or 0.9% saline.

Chemical preparation of cotton:

Common Johnson and Johnson cotton was boiled in 3N KOH for 24 hrs. and then washed to neutrality with distilled water. Any dark specks were removed with forceps after drying in acetone/air at room temperature. The dry fibers were chopped with scissors to lengths of 1 cm. or less and placed in a Spex grinder for 10 min. The powdered cellulose was slurried with 0.5N HCl for a few seconds and then vacuum-filtered through a sintered glass filter to remove traces of iron. The cellulose was washed on the filter 2X with 0.5N HCl and 3X with distilled water. It was then washec 3X with acetone and left on the filter 3-4 min. to dry. The fine white powder was then transferred into a small vial which was labelled

RG:740617 upon receipt.[a]

Chitinous phlogiston suspension (in light mineral oil)

One hundred mg. each of the floats of whole velella, deacetylated velella, and deproteinated velella (all ground to 200-mesh powders) were triturated separately in a glass mortar with light mineral oil N.F., to a final concentration of 5 mg./ml. These suspensions were then more finely divided and uniformly suspended by using a tissue homogenizer and pestle[b] fitted to an electric motor[c] and spun at approximately 1500 RPM. These preparations were then poured into 10-ml parenteral vials, covered with rubber serum caps, and stored at 4°C for one week prior to use. Before injection into animals, these preparations were warmed to room temperature.

Chitin and cotton phlogiston suspensions (in saline):

One hundred mg. each of the whole, deacetylated, and deproteinated materials derived from the floats of Velella lata, from the pen material of Loligo opalescens, and from the shells of the common shrimp, and 100 mg. of cotton (all ground to 200 mesh powders) were placed separately in a 10-ml. graduate cylinder and suspended in 0.9% saline to a final concentration of 10 mg./ml. These suspensions were then transferred into tissue homogenizers with pestles fitted to an electric motor and spun at 1500 R.P.M. When uniform suspensions were achieved the preparations were transferred to 10-ml. parenteral vials with rubber serum caps and stored at 4°C for one

a - R.L. Gainey, Chief Chemist, FMC Corp., Modesto, California.
b - Kontes Glass Co., Vineland, New Jersey. Kontes glass jacketed tissue homogenizer.
c - Talboys Engineering Corp., Emerson, New Jersey, model 106.

week prior to use. Before injection into animals these preparations were warmed to room temperature.

Drug preparation and suspension:

All drugs were suspended by trituration in 0.25% agar[a] and administered orally using a constant 10 ml/kg. dosage volume. Preparation of drug suspension and storage conditions were as follows: aspirin[b], 300 mg./kg., hydrocortisone alcohol[c], 20 mg./kg., phenylbutazone,[d] 100 mg./kg., indomethacin,[e] 10 mg./kg., and chlorpromazine,[f] 100 mg./kg. These solution-suspensions were prepared three days prior to use and stored at 4°C. Cryogenine[g] suspension, 100 mg./kg. was made immediately prior to use. All drugs were warmed to room temperature before being administered to the animals.

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a - Difco Laboratories, Detroit, Michigan; Catalogue #0140-01.
b - Mallinckrodt Chemical Works, St. Louis, Missouri.
c - Nutritional Biochemical Corp. Hollywood, Florida; Control #2912.
d - Ciba Pharmaceutical Co., Summit, New Jersey; lot #SN5273.
e - Merck, Sharp, & Dohme Research Iab., Rahway, New Jersey; lot # 1-590226-00A104.
f - Smith, Kline, & French Lab., Philadelphia, Pennsylvania.
g - Compliments of William Watson (28).

RESULTS

Rat pedal edema assays:

The initial study was performed in order to determine whether or not velella materials were capable of inducing an inflammatory response; and if so, whether this response was similar to that induced by mycobacterium adjuvant. Since killed Mycobacterium butyricum (administered in light mineral oil and injected pedally in rats) induces a chronic long term polyarthritis, which is apparently immunologic in nature (30), all velella materials were likewise suspended in light mineral oil. Futhermore, each of the velella materials was studied at levels identical (mg. for mg.) to those widely used for inducing inflammation with Mycobacterium butyricum.

When Mycobacterium butyricum was suspended in light mineral oil and injected pedally into rats, it produced a statistically significant amount of swelling in the injected paw when compared to the injected paws of animals receiving only the light mineral oil vehicle control. This swelling began at +1 day and persisted through the 20-day observation period, and was statistically significant from the vehicle control at a probability of p 0.001 (see Table I). Whole velella float material and partly deacetylated

Table I: Mean injected paw volumes of rats with adjuvant-induced inflammation or velella-induced inflammation (phlogiston vehicle: light mineral oil)

	N	+5 min. volume, ml.	Change from +5 min. volume, ml. (±1 SEM)					
			+1 hr.	+3 hr.	+1 d.	+2 d.	+3 d.	+4 d.
Light mineral oil vehicle control	8	2.09	-0.09 (0.02)	-0.08 (0.05)	+0.12 (0.05)	+0.06 (0.04)	+0.17 (0.05)	+0.18 (0.07)
Mycobacterium adjuvant	8	2.06	-0.07 (0.04)	+0.06 (0.05)	+1.17^{c} (0.02)	+1.72^{c} (0.14)	+1.91^{c} (0.16)	+1.81^{c} (0.21)
Whole velella float material	8	1.97	-0.08 (0.03)	+0.05^{a} (0.03)	+0.26 (0.09)	+0.19 (0.06)	+0.22 (0.02)	+0.19 (0.06)
Partly deacetylated velella float material	8	1.98	+0.07^{b} (0.04)	+0.11^{b} (0.04)	+0.32^{a} (0.07)	+0.23^{a} (0.07)	+0.28 (0.03)	+0.21 (0.05)
Deproteinated velella float material	8	2.00	-0.11 (0.08)	+0.00 (0.03)	+0.15 (0.08)	+0.13 (0.07)	+0.17 (0.06)	+0.17 (0.06)

^{a}, ^{b}, and ^{c} indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Table I continued.

	Change from +5 min. volume, ml. (±1 SEM)							
	+5 d.	+6 d.	+7 d.	+8 d.	+9 d.	+10 d.	+11 d.	+12 d.
Light mineral oil vehicle control	+0.20 (0.05)	+0.13 (0.05)	+0.12 (0.04)	+0.15 (0.05)	-0.01 (0.00)	-0.03 (0.04)	-0.01 (0.04)	+0.02 (0.05)
Mycobacterium adjuvant	+1.80[c] (0.20)	+1.62[c] (0.20)	+1.48[c] (0.21)	+1.50[c] (0.24)	+1.20[c] (0.25)	+1.30[c] (0.26)	+1.34[c] (0.26)	+1.23[c] (0.32)
Whole velella float material	+0.13 (0.06)	+0.10 (0.06)	+0.14 (0.02)	+0.17 (0.07)	+0.12[b] (0.02)	+0.10[a] (0.03)	+0.20[b] (0.05)	+0.12 (0.05)
Partly deacetylated velella float material	+0.23 (0.05)	+0.19 (0.02)	+0.13 (0.05)	+0.18 (0.04)	+0.14 (0.07)	+0.13[a] (0.04)	+0.10 (0.05)	+0.09 (0.04)
Deproteinated velella float material	+0.11 (0.05)	+0.09 (0.06)	+0.14 (0.03)	+0.07 (0.06)	+0.12 (0.05)	+0.08 (0.07)	+0.00 (0.05)	+0.01 (0.06)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values $p > 0.05$.

Table I continued.

	Change from +5 min. volume, ml. (1 SEM)							
	+13 d.	+14 d.	+15 d.	+16 d.	+17 d.	+18 d.	+19 d.	+20 d.
Light mineral oil vehicle control	-0.01 (0.05)	-0.02 (0.04)	+0.04 (0.04)	+0.01 (0.06)	+0.04 (0.05)	-0.02 (0.05)	-0.03 (0.06)	+0.05 (0.06)
Mycobacterium adjuvant	+1.53[c] (0.27)	+1.70[c] (0.29)	+1.68[c] (0.31)	+1.75[c] (0.29)	+1.77[c] (0.28)	+1.79[c] (0.39)	+1.93[c] (0.29)	+2.03[c] (0.30)
Whole velella float material	+0.16[a] (0.05)	+0.17[a] (0.05)	+0.18[a] (0.05)	+0.14 (0.06)	+0.19 (0.04)	+0.19[a] (0.04)	+0.17[a] (0.04)	+0.25[a] (0.07)
Partly deacetylated velella float material	+0.12 (0.05)	+0.10 (0.05)	+0.16 (0.05)	+0.17 (0.07)	+0.20 (0.07)	+0.23[b] (0.07)	+0.16 (0.08)	+0.13 (0.07)
Deproteinated velella float material	+0.04 (0.07)	+0.15 (0.07)	+0.13 (0.06)	+0.10 (0.07)	+0.14 (0.07)	+0.11 (0.05)	+0.19 (0.08)	+0.13 (0.05)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values $p > 0.05$.

velella float material also produced some swelling in the injected paws which was statistically differnt from the control, but the time-sequence of the swelling did not appear to follow any consistent pattern. Futhermore, the swelling produced by both materials was only about one-sixth that produced by the Mycobacterium butyricum (see Table I and Figures 1-2). Deproteinated velella float material did not produce any statistically significant degree of swelling (see Table I and Figure 3). There was no evidence of the slow onset of polyarthritis in the contralateral paws of animals injected with velella materials as was seen in those animals receiving the mycobacterium adjuvant (see Table II and Figures 4-6).

The results shown in Tables III and IV, and in Figures 7-13, are from a study which was performed in order to determine whether or not the velella materials were capable of producing an acute, short-term inflammation similar to that produced by carrageenin (31). Therefore, all velella materials, like carrageenin, were suspended in 0.9% saline and studied at levels identical (mg. for mg.) to those widely used for inducing inflammation with carrageenin (31).

When carrageenin was suspended in 0.9% saline and injected pedally into rats, it produced a rapid-onset, short-term inflammation in the injected paw as expected. A peak in swelling was reached at +3 hr., which was statistically significantly different from the saline vehicle control at a probability level of $p < 0.001$. This swelling disappeared and was no longer statistically significantly different from the saline vehicle control by +1 day (see Table III). The velella materials, when suspended in 0.9% saline, also produced a rapid onset, carrageenin-like inflammation, but this inflammation was not as intense as that seen with carrageenin. Futhermore, this

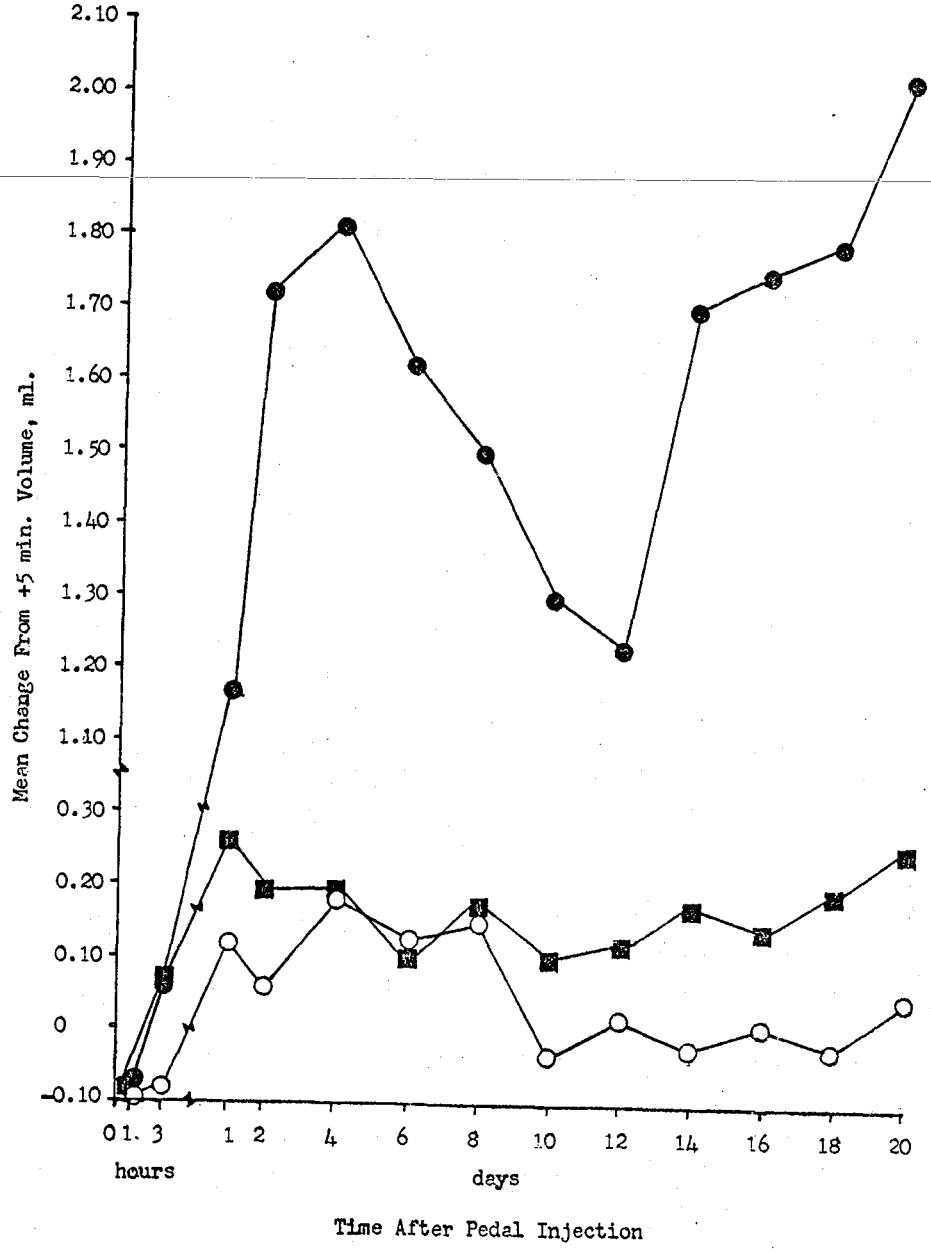
Fig. 1 -- A comparison of the types of inflammation induced in the injected paws of animals by mycobacterium adjuvant and whole velella float material.
(phlogiston vehicle: light mineral oil)

Values indicate change from +5 min. volume, ml.

● mycobacterium adjuvant

■ whole velella float material

○ light mineral oil control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1.20
1.10
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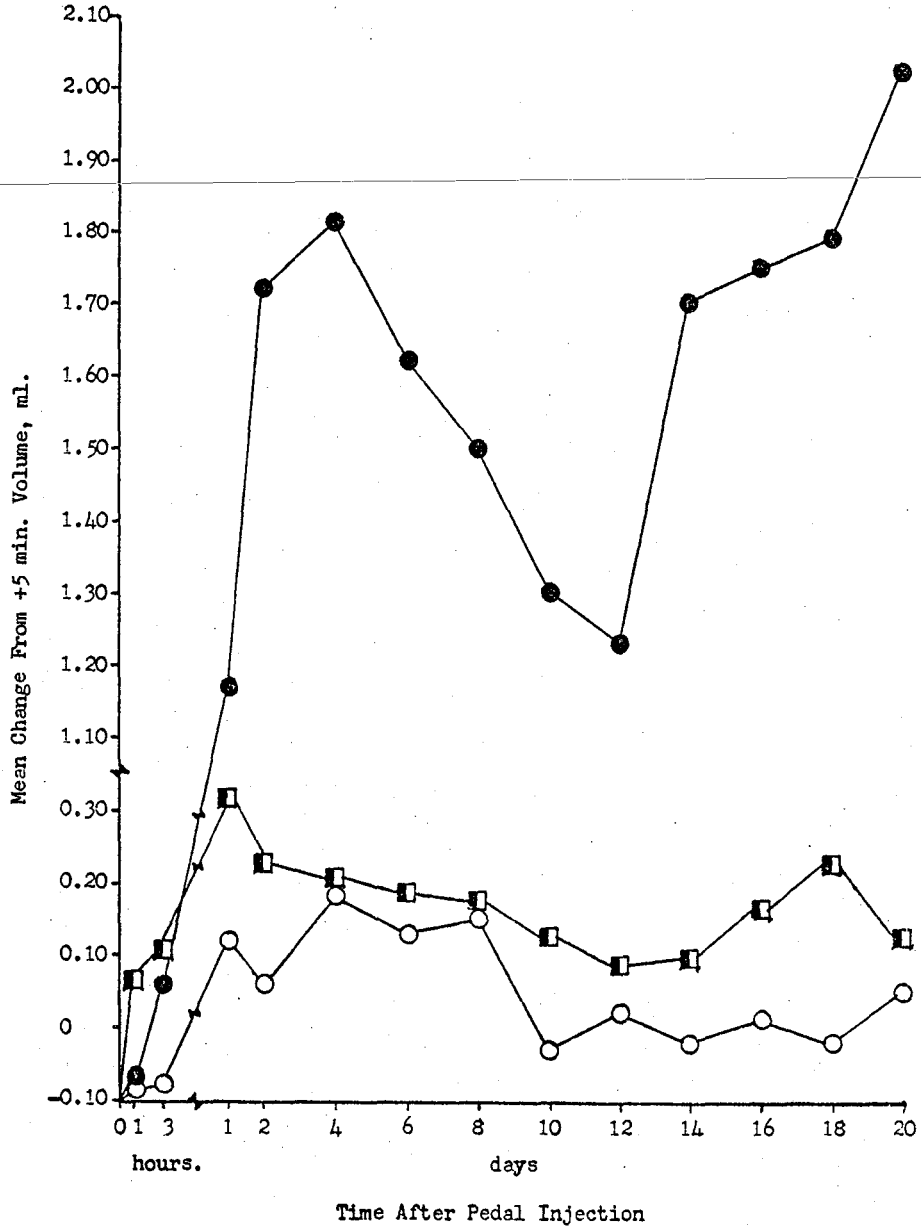
0.10
0
-0.10
Mean Change From +5 min. Volume, ml.
0 1. 3 1 2 4 6 8 10 12 14 16 18 20
hours
days
Time After Pedal Injection

Fig. 2 -- A comparison of the types of inflammation induced in the injected paws of animals by mycobacterium adjuvant and partly deacetylated velella float material.

(phlogiston vehicle: light mineral oil)

Values indicate change from +5 min. volume, ml.

- mycobacterium adjuvant
- partly deacetylated velella
- light mineral oil control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2.00
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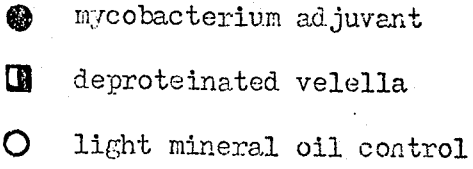
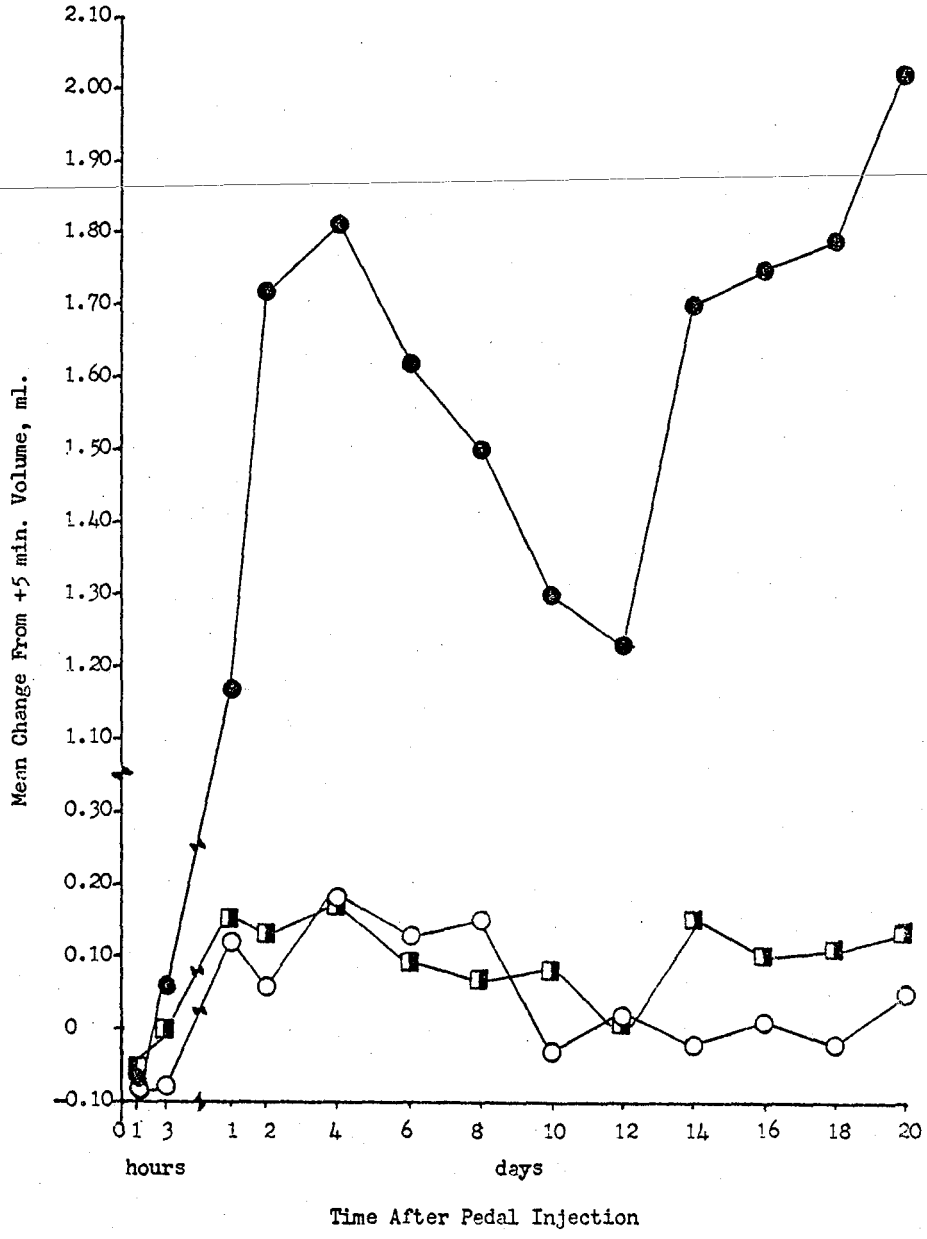
0.20
0.10
0
-0.10
Mean Change From +5 min. Volume, ml.
0 1 3 1 2 4 6 8 10 12 14 16 18 20
hours.
days
Time After Pedal Injection

Fig. 3 -- A comparison of the types of inflammation induced in the injected paws of animals by mycobacterium adjuvant and deproteinated velella float material. (phlogiston vehicle: light mineral oil)

Values indicate change from +5 min. volume, ml.




2.10
2.00
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1.30
1.20
1.10
0.30
0.20
0.10
0
-0.10
Mean Change From +5 min. Volume, ml.
0 1 3 1 2 4 6 8 10 12 14 16 18 20
hours
days
Time After Pedal Injection

Table II: Mean contralateral paw volumes of rats with adjuvant-induced inflammation or velella-induced inflammation (phlogiston vehicle: light mineral oil)

	N	+5 min. volume, ml.	Change from +5 min. volume, ml. (± 1 SEM)					
			+1 hr.	+3 hr.	+1 d.	+2 d.	+3 d.	+4 d.
Light mineral oil vehicle control	8	1.88	-0.01 (0.03)	-0.03 (0.05)	+0.05 (0.04)	-0.06 (0.03)	+0.10 (0.08)	+0.00 (0.05)
Mycobacterium adjuvant	8	1.87	-0.08 (0.04)	-0.03 (0.05)	-0.09 (0.09)	-0.12 (0.05)	+0.02 (0.03)	-0.08 (0.03)
Whole velella float material	8	1.77	-0.06 (0.04)	-0.01 (0.03)	+0.08 (0.06)	+0.00 (0.06)	+0.05 (0.03)	-0.04 (0.06)
Partly deacetylated velella float material	8	1.74	+0.09[a] (0.04)	+0.05 (0.05)	+0.08 (0.06)	+0.06 (0.07)	+0.12 (0.03)	+0.06 (0.06)
Deproteinated velella float material	8	1.75	+0.08 (0.04)	+0.00 (0.02)	+0.01 (0.03)	+0.11[a] (0.07)	+0.11 (0.04)	+0.07 (0.03)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values $p > 0.05$.

Table II continued.

	Change from +5 min. volume, ml. (1 SEM)							
	+5 d.	+6 d.	+7 d.	+8 d.	+9 d.	+10 d.	+11 d.	+12 d.
Light mineral oil vehicle control	+0.04 (0.02)	+0.04 (0.04)	+0.03 (0.04)	+0.06 (0.04)	-0.03 (0.04)	-0.04 (0.03)	-0.03 (0.02)	-0.02 (0.04)
Mycobacterium adjuvant	-0.01 (0.04)	-0.02 (0.03)	-0.06 (0.05)	-0.07 (0.06)	-0.08 (0.06)	-0.14 (0.06)	-0.13[b] (0.03)	-0.15 (0.05)
Whole velella float material	-0.01 (0.05)	-0.01 (0.05)	+0.02 (0.04)	+0.08 (0.09)	+0.07 (0.04)	+0.03 (0.04)	+0.03 (0.05)	+0.02 (0.05)
Partly deacetylated velella float material	+0.08 (0.03)	+0.14 (0.04)	+0.03 (0.04)	+0.06 (0.04)	+0.07 (0.06)	+0.07 (0.03)	+0.02 (0.04)	+0.06 (0.04)
Deproteinated velella float material	+0.08 (0.03)	+0.09 (0.03)	+0.09 (0.04)	+0.06 (0.03)	+0.08[a] (0.03)	+0.03 (0.03)	+0.05 (0.04)	+0.02 (0.03)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ respectively. For all other values, $p > 0.05$.

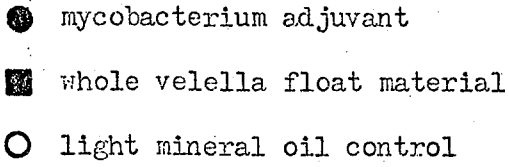
Table II continued.

	Change from +5 min. volume, ml. (1 SEM)							
	+13 d.	+14 d.	+15 d.	+16 d.	+17 d.	+18 d.	+19 d.	+20 d.
Light mineral oil vehicle control	-0.04 (0.04)	-0.03 (0.06)	+0.01 (0.03)	-0.01 (0.06)	+0.00 (0.03)	-0.04 (0.03)	-0.07 (0.05)	-0.01 (0.04)
Mycobacterium adjuvant	-0.01 (0.03)	+0.03 (0.04)	+0.15 (0.12)	+0.38 (0.20)	+0.43 (0.27)	+0.90[b] (0.34)	+0.82[a] (0.33)	+0.74 (0.31)
Whole velella float material	+0.10 (0.05)	+0.08 (0.05)	+0.09 (0.03)	+0.05 (0.05)	+0.08 (0.04)	+0.08 (0.05)	+0.10[a] (0.03)	+0.08 (0.31)
Partly deacetylated velella float material	+0.10 (0.05)	+0.11 (0.06)	+0.13 (0.05)	+0.10 (0.05)	+0.13 (0.04)	+0.15[b] (0.04)	+0.06 (0.06)	+0.09 (0.05)
Deproteinated velella float material	+0.10 (0.05)	+0.09 (0.05)	+0.12 (0.04)	+0.11 (0.04)	+0.13 (0.04)	+0.10[a] (0.04)	+0.09[a] (0.05)	+0.14[a] (0.04)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Fig. 4 -- A comparison of the types of inflammation induced in the contralateral paws of animals by mycobacterium adjuvant and whole velella float material.
(phlogiston vehicle: light mineral oil)

Values indicate change from +5 min. volume, ml.



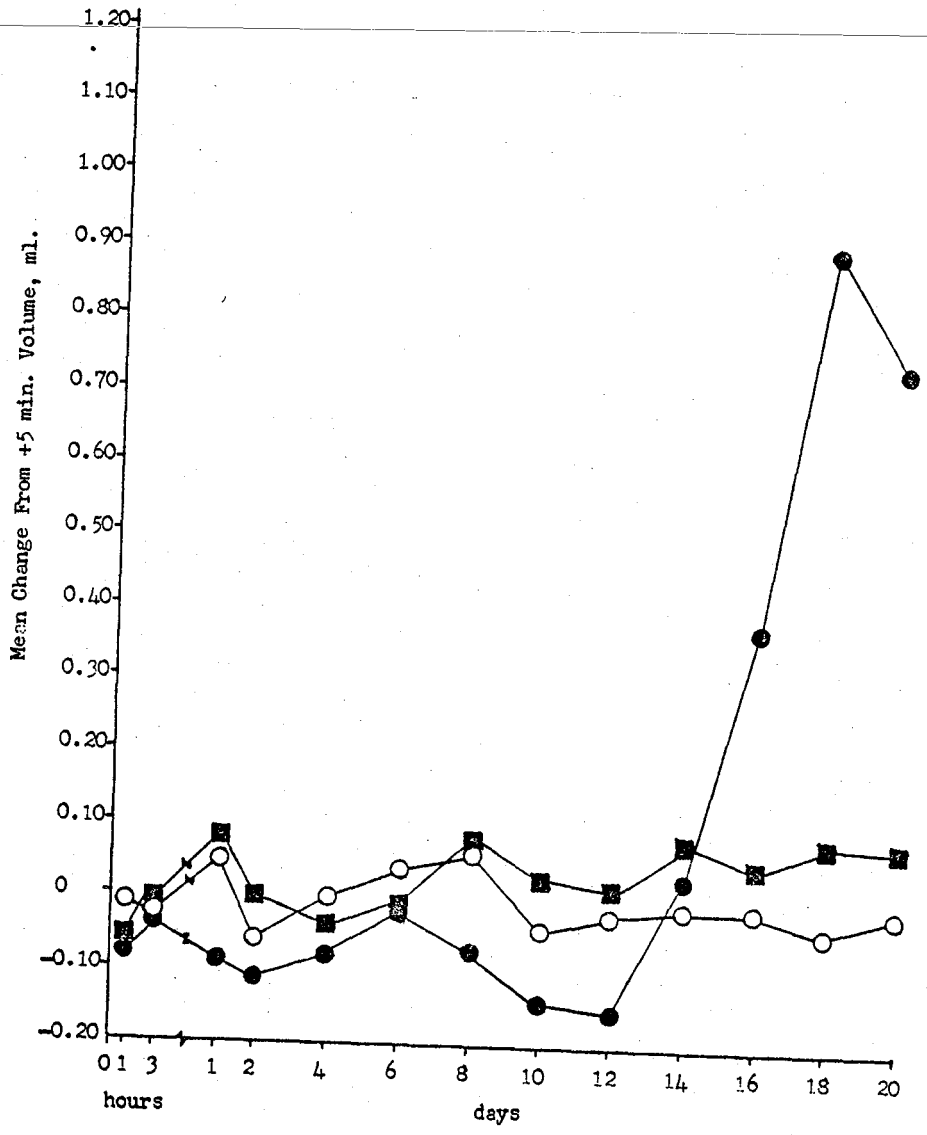

Mean Change From +5 min. Volume, ml.
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
-0.20
0 1 3 1 2 4 6 8 10 12 14 16 18 20
hours
days
Time After Pedal Injection

Fig. 5 -- A comparison of the types of inflammation induced in the contralateral paws of animals by mycobacterium adjuvant and partly deacetylated velella float material. (phlogiston vehicle: light mineral oil)

Values indicate change from +5 min. volume, ml.

- mycobacterium adjuvant
- partly deacetylated velella
- light mineral oil

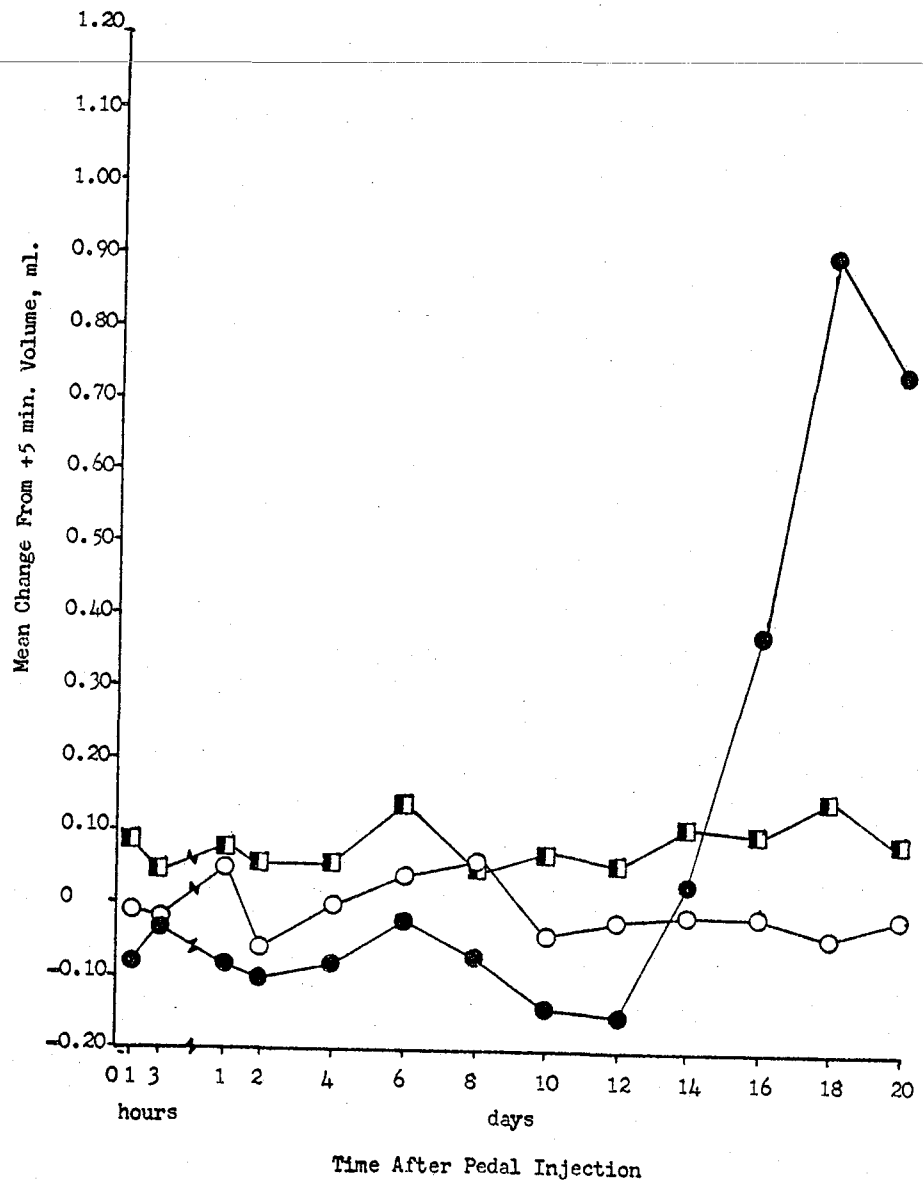

1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
-0.20
Mean Change From +5 min. Volume, ml.
0 1 3 1 2 4 6 8 10 12 14 16 18 20
hours
days
Time After Pedal Injection

Fig. 6 -- A comparison of the types of inflammation induced in the contralateral paws of animals by mycobacterium adjuvant and deproteinated velella float material. (phlogiston vehicle: light mineral oil)

Values indicate change from +5 min. volume, ml.

● mycobacterium adjuvant

◧ deproteinated velella

○ light mineral oil

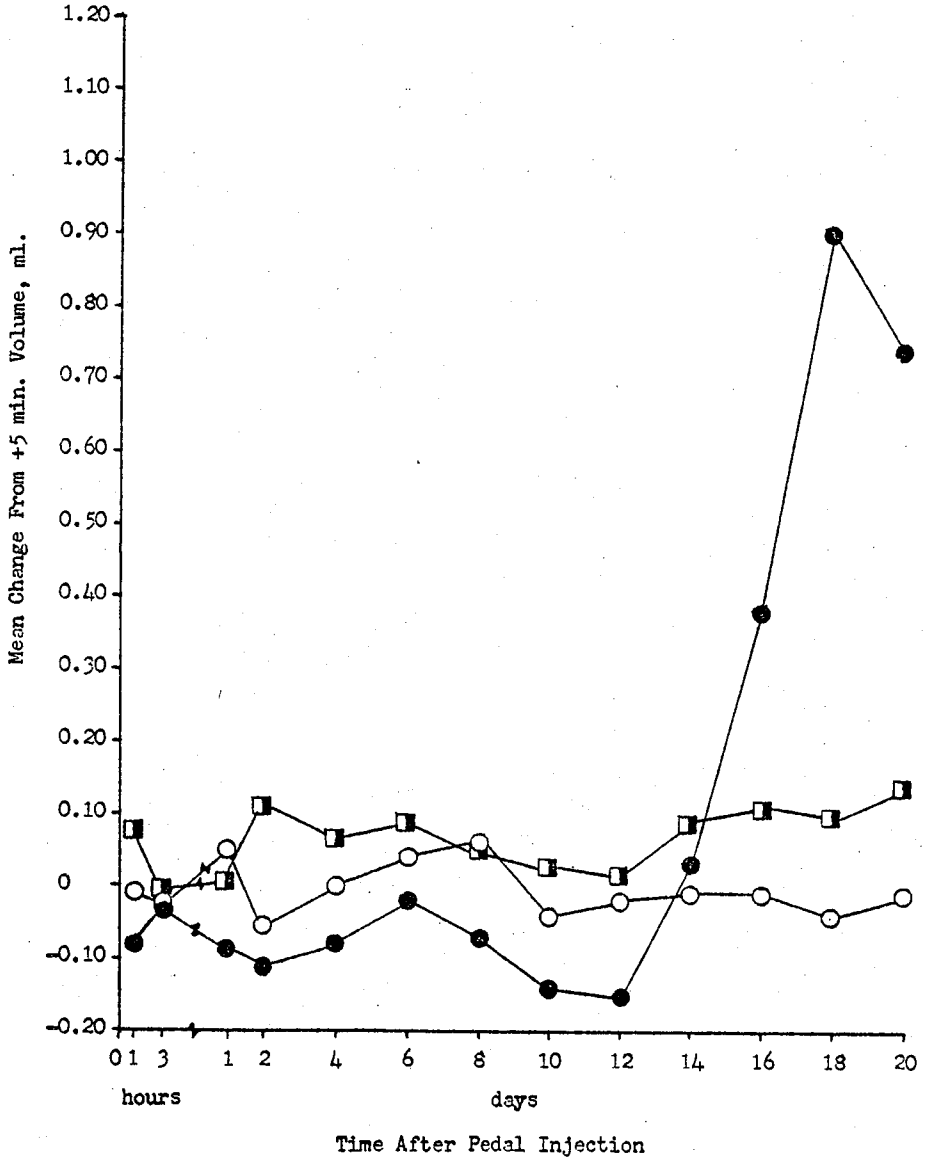

1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
-0.20
Mean Change From +5 min. Volume, ml.
0 1 3 1 2 4 6 8 10 12 14 16 18 20
hours
days
Time After Pedal Injection

Table III: Mean injected paw volumes of rats with carrageenin-induced inflammation or velella-induced inflammation (phlogiston vehicle: 0.9% saline)

	N	+5 min. volume, ml.	Change from +5 min. volume, ml. (±1SEM)					
			+1 hr.	+3 hr.	+1 d.	+2 d.	+3 d.	+4 d.
0.9% Saline vehicle control	8	1.82	+0.03 (0.09)	-0.02 (0.03)	+0.10 (0.08)	-0.03 (0.06)	-0.04 (0.04)	-0.12 (0.05)
Carrageenin	8	1.96	+0.13 (0.07)	+0.72^{c} (0.08)	+0.19 (0.06)	-0.01 (0.04)	+0.04 (0.07)	-0.05 (0.04)
Whole velella float material	8	2.01	-0.05 (0.04)	+0.20^{b} (0.04)	+0.51^{b} (0.06)	+0.25^{b} (0.03)	+0.23^{c} (0.03)	+0.11^{b} (0.03)
Partly deacetylated velella float material	8	2.03	+0.04 (0.06)	+0.23^{b} (0.06)	+0.36^{a} (0.09)	+0.14 (0.06)	+0.10 (0.07)	+0.09^{a} (0.08)
Deproteinated velella float material	8	1.89	+0.03 (0.07)	+0.08 (0.08)	+0.29 (0.08)	+0.14 (0.06)	+0.10 (0.09)	+0.08 (0.08)

^{a}, ^{b}, and ^{c} indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Table III continued.

	Change from +5 min. volume, ml. (±1 SEM)							
	+5 d.	+6 d.	+7 d.	+8 d.	+9 d.	+10 d.	+11 d.	+12 d.
0.9% Saline vehicle control	-0.11 (0.04)	-0.09 (0.04)	-0.10 (0.02)	+0.02 (0.09)	-0.11 (0.03)	-0.07 (0.02)	-0.11 (0.03)	-0.01 (0.03)
Carrageenin	-0.01 (0.02)	+0.01 (0.03)	-0.12 (0.03)	-0.09 (0.01)	-0.04 (0.03)	-0.12 (0.01)	-0.11 (0.04)	-0.07 (0.04)
Whole velella float material	+0.12[b] (0.03)	+0.10[a] (0.06)	+0.14[b] (0.09)	+0.10 (0.06)	+0.07 (0.04)	+0.06 (0.07)	+0.04[a] (0.09)	+0.03 (0.07)
Partly deacetylated velella float material	+0.11[b] (0.05)	+0.09[a] (0.06)	+0.11[b] (0.05)	+0.09 (0.07)	+0.09[a] (0.08)	+0.05 (0.06)	+0.04[a] (0.05)	+0.03 (0.03)
Deproteinated velella float material	+0.05[a] (0.05)	+0.09[a] (0.06)	+0.12[b] (0.07)	+0.10 (0.08)	+0.07 (0.07)	+0.05 (0.07)	+0.01[a] (0.05)	+0.07 (0.05)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Table III continued.

	Change from +5 min. volume, ml. (±1 SEM)							
	+13 d.	+14 d.	+15 d.	+16 d.	+17 d.	+18 d.	+19 d.	+20 d.
0.9% Saline vehicle control	-0.10 (0.04)	-0.04 (0.05)	-0.03 (0.05)	-0.07 (0.04)	-0.04 (0.03)	-0.03 (0.03)	-0.08 (0.03)	+0.02 (0.04)
Carrageenin	-0.08 (0.05)	-0.08 (0.03)	-0.02 (0.03)	-0.10 (0.04)	-0.07 (0.03)	-0.08 (0.04)	-0.06 (0.03)	+0.01 (0.06)
Whole velella float material	+0.05 (0.07)	+0.07 (0.06)	+0.10 (0.05)	+0.07 (0.07)	+0.08 (0.06)	+0.03 (0.05)	+0.05 (0.07)	+0.07 (0.05)
Partly deacetylated velella float material	+0.05[a] (0.05)	+0.13 (0.05)	+0.08 (0.04)	+0.14[c] (0.04)	+0.15[c] (0.03)	+0.07[a] (0.05)	+0.10[a] (0.06)	+0.04 (0.06)
Deproteinated velella float material	+0.10[a] (0.07)	+0.12 (0.06)	+0.07 (0.05)	+0.12[a] (0.07)	+0.14[a] (0.06)	+0.08[a] (0.06)	+0.10[a] (0.06)	+0.09 (0.05)

a, b, and c indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Table IV: Mean contralateral paw volumes of rats with carrageenin-induced inflammation or velella-induced inflammation (phlogiston vehicle: 0.9% saline)

	N	+5 min. volume, ml.	Change from +5 min. volume, ml. (± 1SEM)					
			+1 hr.	+3 hr.	+1 d.	+2 d.	+3 d.	+4 d.
0.9% Saline vehicle control	8	1.60	-0.02 (0.04)	+0.03 (0.03)	+0.20 (0.03)	+0.19 (0.07)	+0.13 (0.02)	+0.05 (0.03)
Carrageenin	8	1.67	-0.03 (0.03)	+0.13 (0.04)	+0.13 (0.04)	+0.04 (0.05)	+0.06 (0.04)	+0.04 (0.03)
Whole velella float material	8	1.70	+0.00 (0.05)	+0.08 (0.05)	+0.14 (0.05)	+0.07 (0.05)	+0.13 (0.03)	-0.02 (0.04)
Partly deacetylated velella float material	8	1.76	+0.03 (0.03)	+0.10 (0.03)	+0.08 (0.05)	+0.13 (0.05)	+0.11 (0.07)	+0.10 (0.04)
Deproteinated velella float material	8	1.63	+0.05 (0.03)	+0.10 (0.02)	+0.09 (0.05)	+0.10 (0.04)	+0.10 (0.06)	+0.04 (0.05)

a, b, and c indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Table IV continued.

	Change from +5 min. volume, ml. (±1 SEM)							
	+5 d.	+6 d.	+7 d.	+8 d.	+9 d.	+10 d.	+11 d.	+12 d.
0.9% Saline vehicle control	+0.08 (0.05)	+0.08 (0.03)	+0.08 (0.02)	+0.08 (0.03)	+0.07 (0.04)	+0.12 (0.02)	+0.08 (0.02)	+0.09 (0.03)
Carrageenin	+0.07 (0.01)	+0.10 (0.04)	+0.03 (0.02)	+0.05 (0.03)	+0.10 (0.03)	+0.05[a] (0.02)	+0.05 (0.03)	+0.04 (0.02)
Whole velella float material	+0.07 (0.02)	+0.07 (0.04)	+0.06 (0.05)	+0.05 (0.03)	+0.11 (0.05)	+0.08 (0.04)	+0.05 (0.06)	+0.06 (0.04)
Partly deacetylated velella float material	+0.13 (0.04)	+0.10 (0.04)	+0.13 (0.05)	+0.13 (0.03)	+0.15 (0.05)	+0.14 (0.05)	+0.15 (0.06)	+0.13 (0.03)
Deproteinated velella float material	+0.11 (0.03)	+0.08 (0.03)	+0.12 (0.04)	+0.00 (0.08)	+0.07 (0.04)	+0.10 (0.05)	+0.09 (0.04)	+0.14 (0.04)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Table IV continued.

	Change from +5 min. volume, ml. (±1 SEM)							
	+13 d.	+14 d.	+15 d.	+16 d.	+17 d.	+18 d.	+19 d.	+20 d.
0.9% Saline vehicle control	+0.06 (0.04)	+0.12 (0.04)	+0.13 (0.02)	+0.09 (0.04)	+0.15 (0.02)	+0.10 (0.03)	+0.10 (0.03)	+0.17 (0.03)
Carrageenin	+0.09 (0.03)	+0.10 (0.03)	+0.19 (0.04)	+0.09 (0.04)	+0.11 (0.04)	+0.04 (0.02)	+0.09 (0.03)	+0.18 (0.06)
Whole velella float material	+0.11 (0.04)	+0.10 (0.04)	+0.13 (0.04)	+0.13 (0.04)	+0.13 (0.04)	+0.10 (0.06)	+0.08 (0.04)	+0.14 (0.03)
Partly deacetylated velella float material	+0.17 (0.05)	+0.14 (0.04)	+0.17 (0.05)	+0.18 (0.05)	+0.21 (0.04)	+0.11 (0.03)	+0.15 (0.05)	+0.15 (0.06)
Deproteinated velella float material	+0.13 (0.05)	+0.15 (0.05)	+0.10 (0.04)	+0.14 (0.04)	+0.21 (0.04)	+0.14 (0.05)	+0.14 (0.05)	+0.15 (0.04)

$\underline{a}$, $\underline{b}$, and $\underline{c}$ indicate $\underline{p} < 0.05$, $\underline{p} < 0.01$, and $\underline{p} < 0.001$, respectively. For all other values, $\underline{p} > 0.05$.

Fig. 7 -- A comparison of the types of inflammation induced in the injected paws of animals by carrageenin and whole velella float material.
(phlogiston vehicle: 0.9% saline)

Values indicate change from +5 min. volume, ml.

● carrageenin

■ whole velella float material

○ 0.9% saline

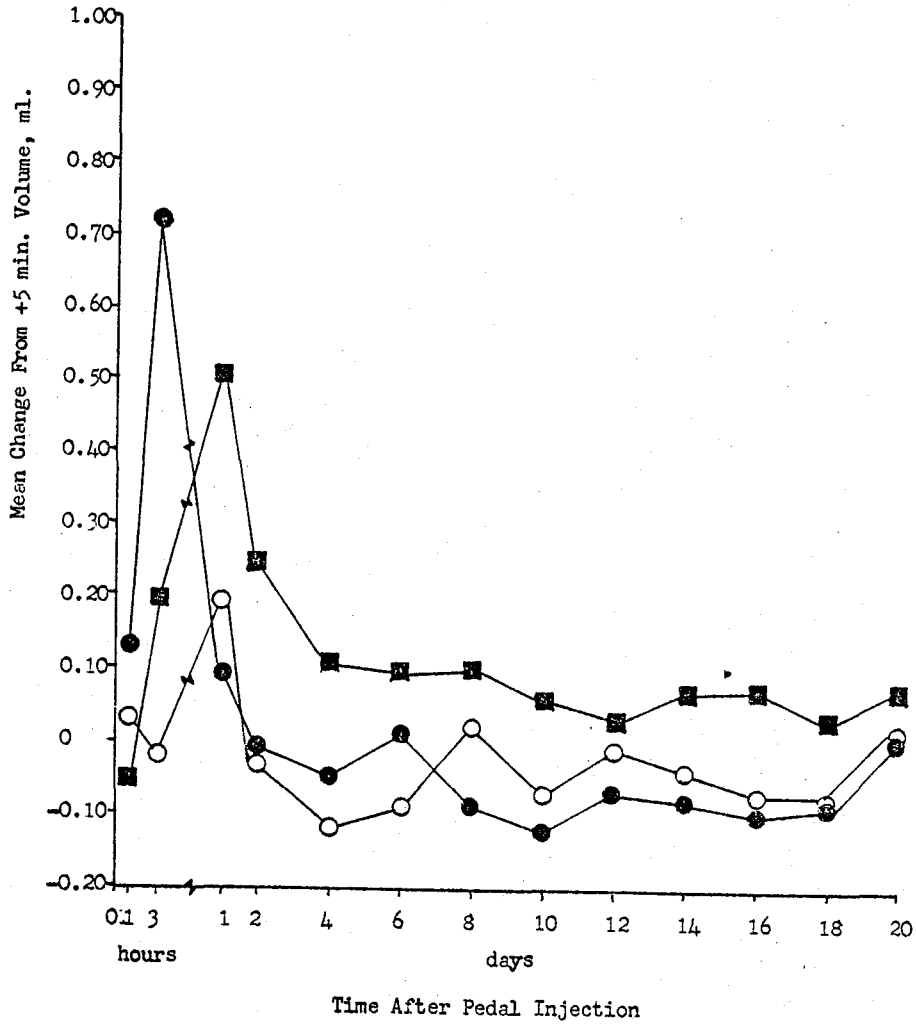

Mean Change From +5 min. Volume, ml.
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
-0.20
0 1 3
hours
1 2 4 6 8 10 12 14 16 18 20
days
Time After Pedal Injection

Fig. 8 -- A comparison of the types of inflammation induced in the injected paws of animals by carrageenin and partly deacetylated velella float material.
(phlogiston vehicle: 0.9% saline)

Values indicate change from +5 min. volume, ml.

● carrageenin

◧ deacetylated velella

○ 0.9% saline

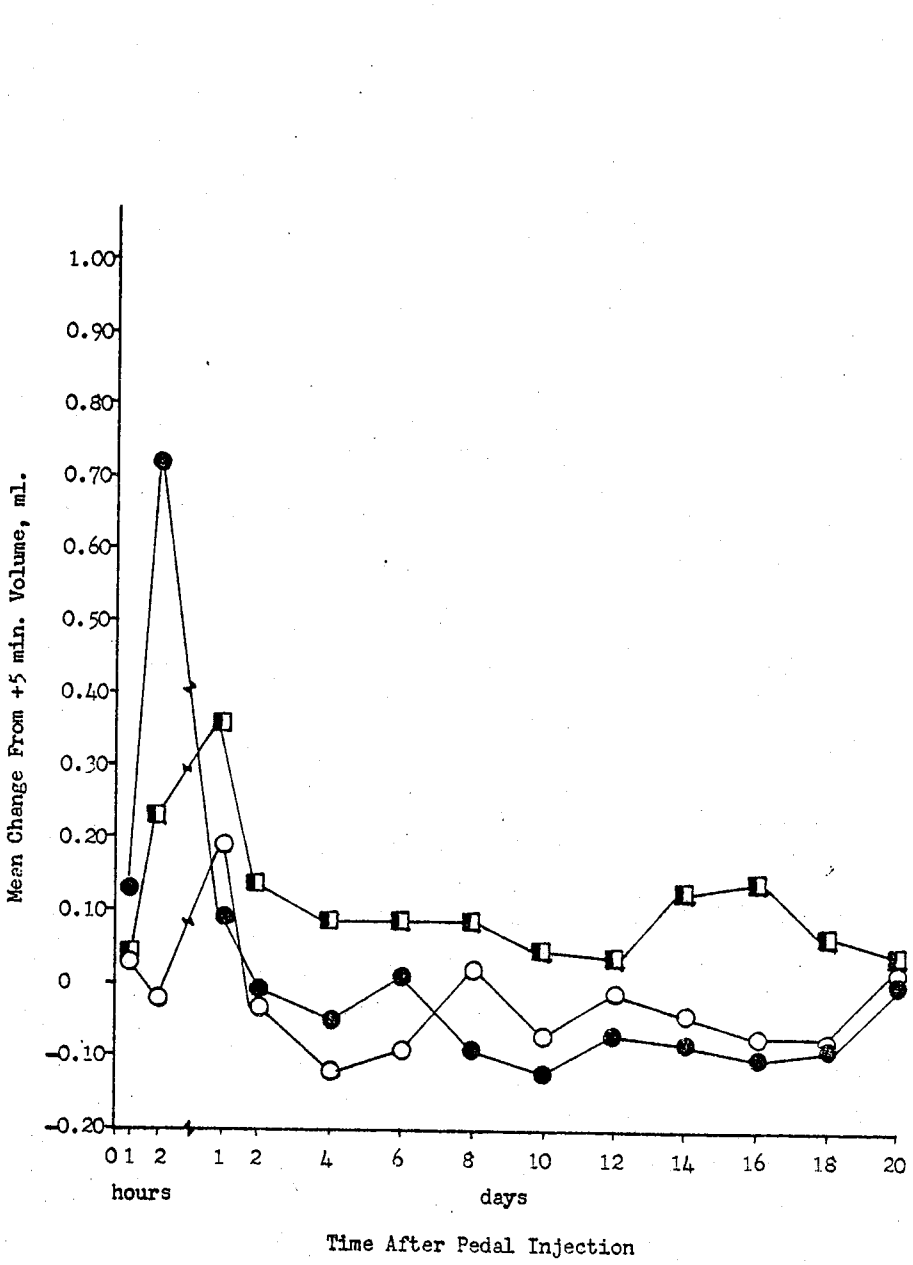

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
-0.20
Mean Change From +5 min. Volume, ml.
0 1 2 1 2 4 6 8 10 12 14 16 18 20
hours
days
Time After Pedal Injection

Fig. 9 -- A comparison of the types of inflammation induced in the injected paws of animals by carrageenin and deproteinated velella float material.
(phlogiston vehicle: 0.9% saline)

Values indicate change from +5 min. volume, ml.

● carrageenin

◧ deproteinated velella

○ 0.9% saline

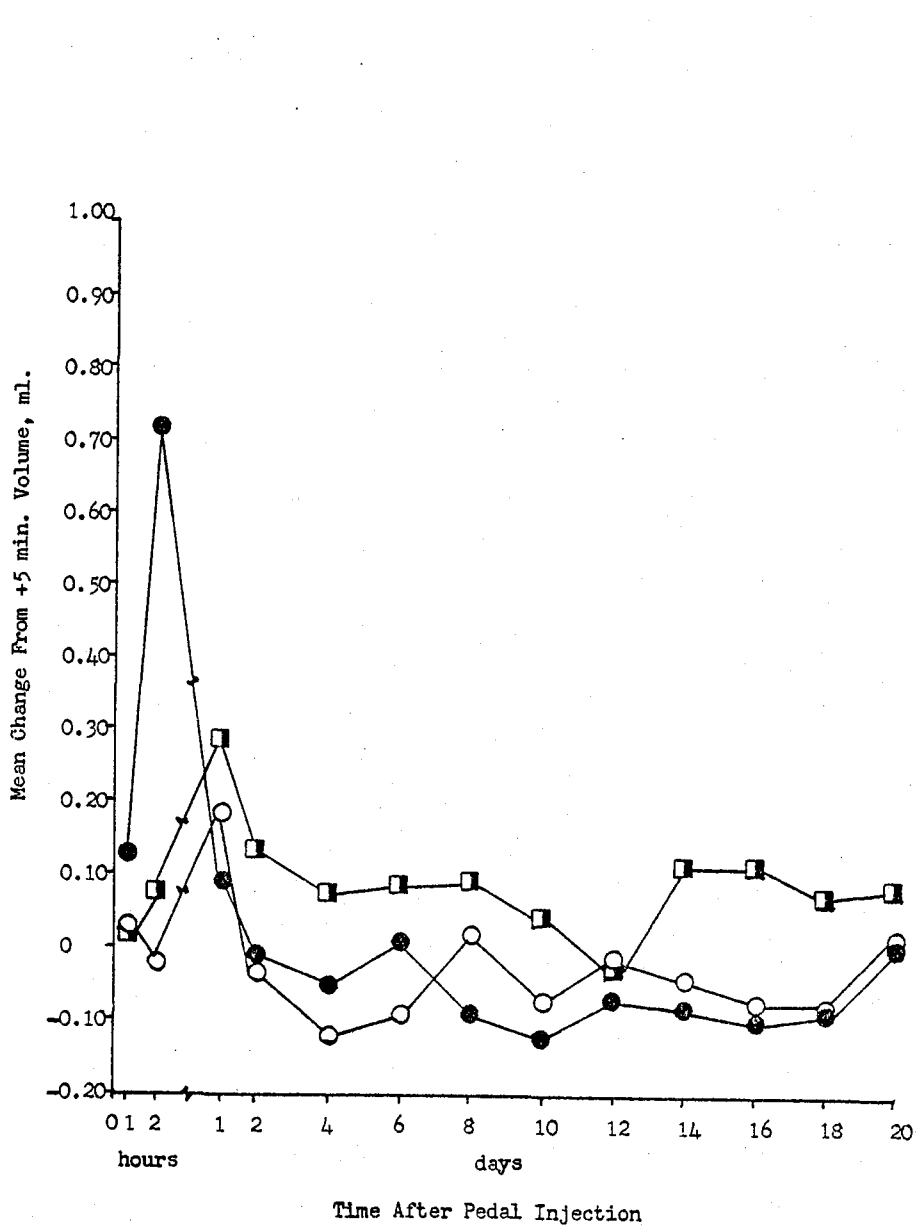
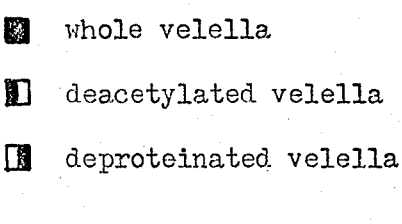

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
-0.20
Mean Change From +5 min. Volume, ml.
0 1 2
1 2 4 6 8 10 12 14 16 18 20
hours
days
Time After Pedal Injection

Fig. 10 -- A comparison of the types of inflammation induced in the injected paws of animals by whole, deacetylated, and deproteinated velella.
(phlogiston vehicle: 0.9% saline)

Values indicate change from +5 min. volume, ml.



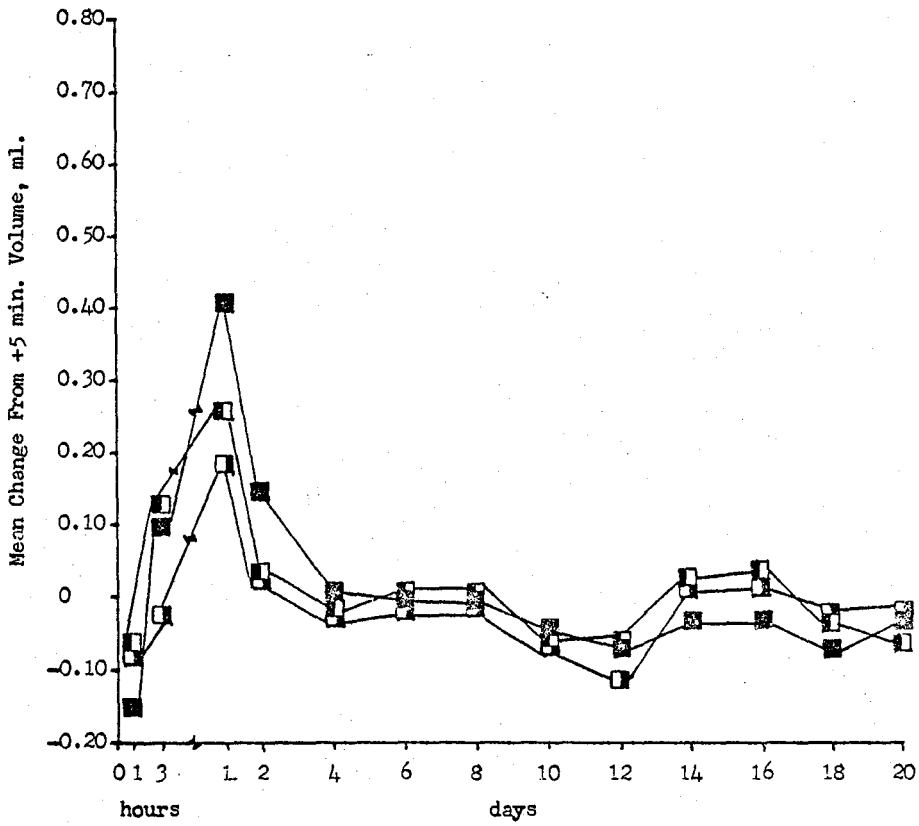

0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
-0.20
Mean Change From +5 min. Volume, ml.
0 1 3
1 2 4 6 8 10 12 14 16 18 20
hours
days
Time After Pedal Injection

Fig. 11 -- A comparison of the types of inflammation induced in the contralateral paws of animals by carrageenin and whole velella float material.
(phlogiston vehicle: 0.9% saline)

Values indicate change from +5 min. volume, ml.

● carrageenin

■ whole velella

○ 0.9% saline

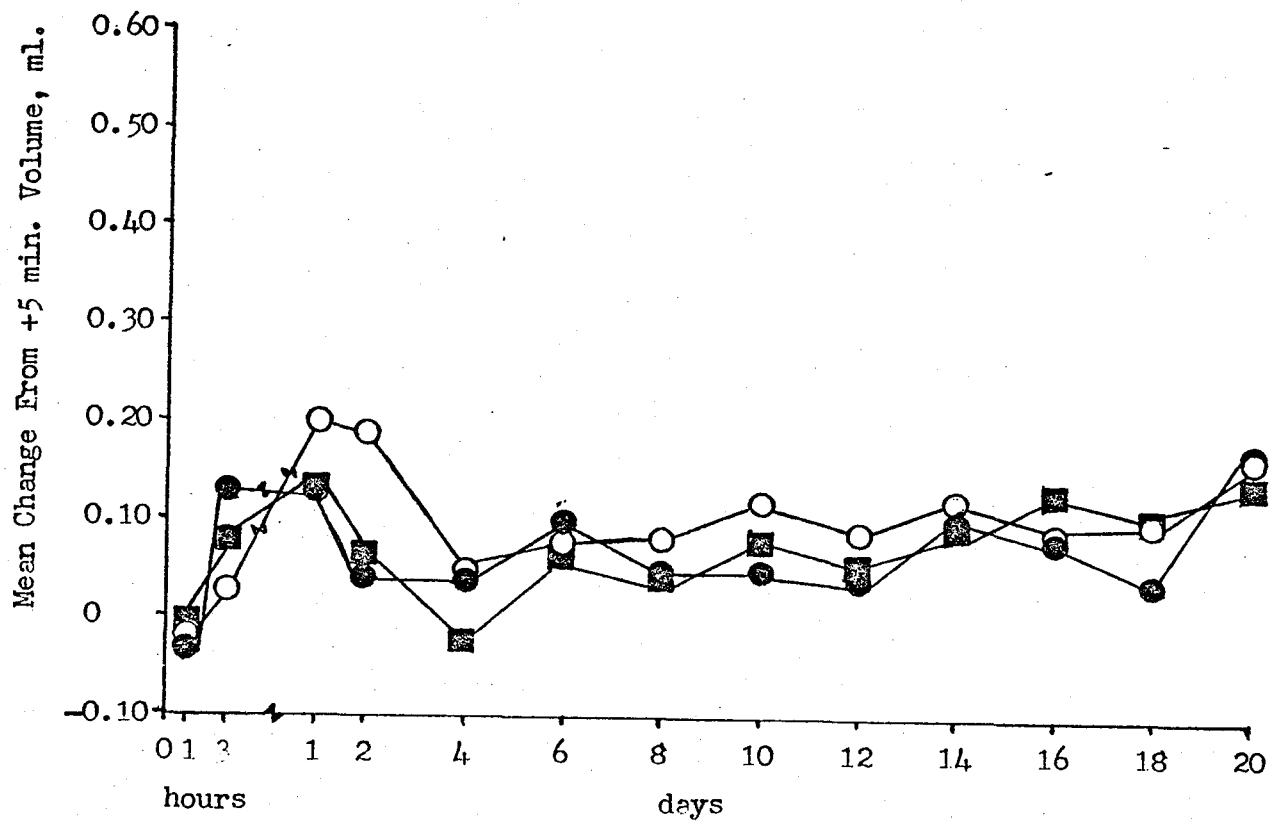

Mean Change From +5 min. Volume, ml.
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
0 1 3
1 2 4 6 8 10 12 14 16 18 20
hours
days
Time After Pedal Injection

Fig. 12 -- A comparison of the types of inflammation induced in the contralateral paws of animals by carrageenin and partly deacetylated velella material.
(phlogiston vehicle: 0.9% saline)

Values indicate change from +5 min. volume, ml.

● carrageenin

▣ deacetylated velella

○ 0.9% saline

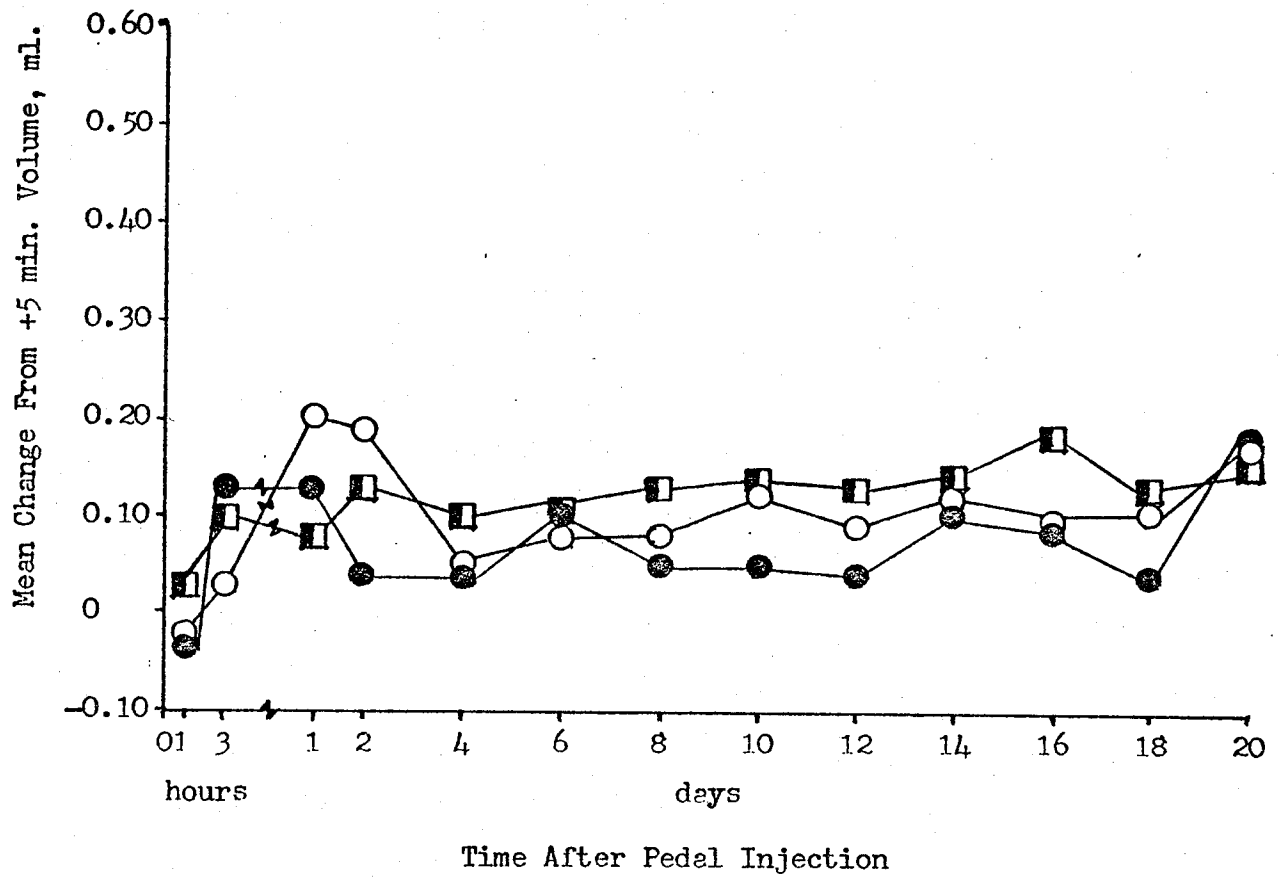

Mean Change From +5 min. Volume, ml.
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
01
3
1
2
4
6
8
10
12
14
16
18
20
hours
days
Time After Pedal Injection

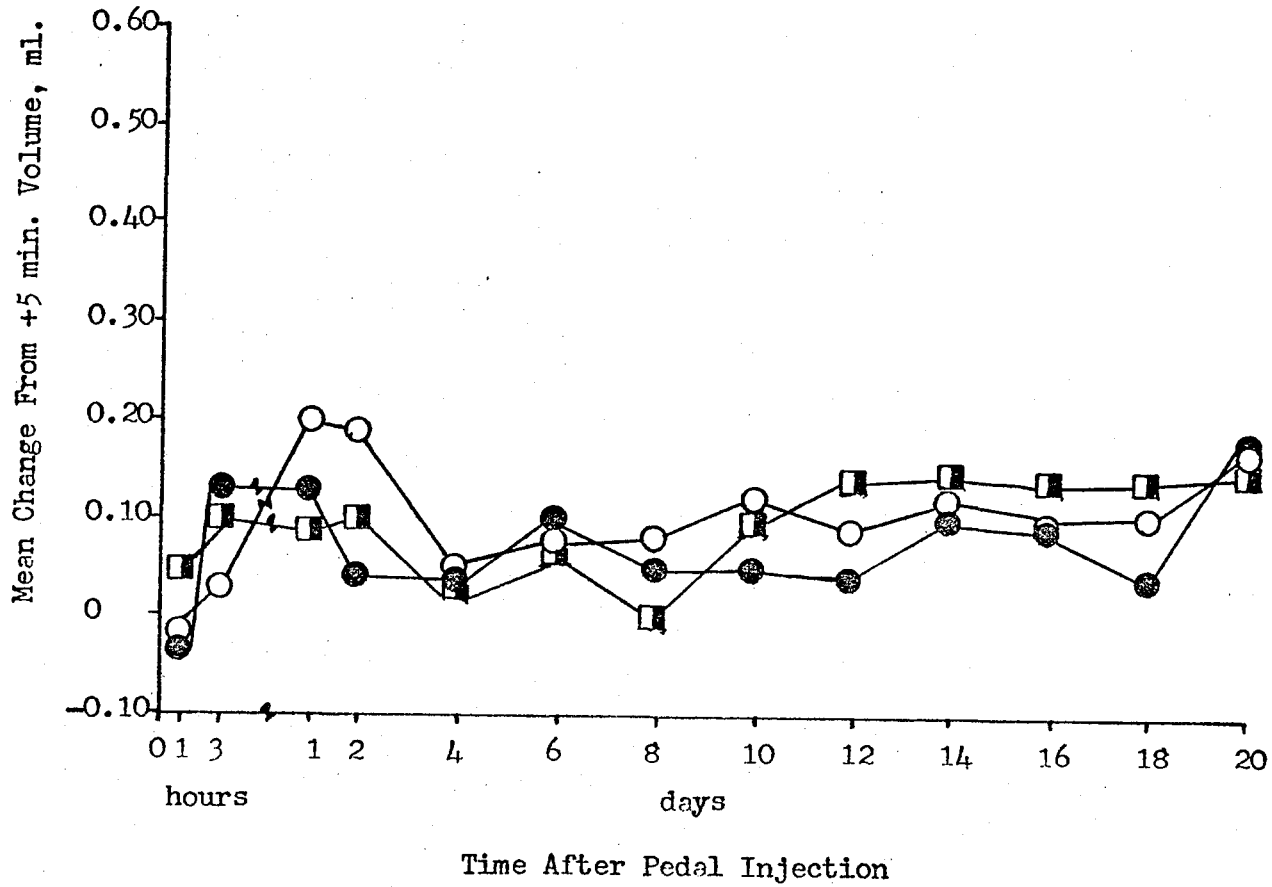
Fig. 13 -- A comparison of the types of inflammation induced in the contralateral paws of animals by carrageenin and deproteinated velella material.
(phlogiston vehicle: 0.9% saline)

Values indicate change from +5 min. volume, ml.

● carrageenin

▮ deproteinated velella

○ 0.9% saline


Mean Change From +5 min. Volume, ml.
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
0 1 3
1 2
4
6
8
10
12
14
16
18
20
hours
days
Time After Pedal Injection

inflammation persisted (unlike carrageenin) through the +20 day observation period (see Table II and Figures 7-9). On an equal weight basis and considering the +1 to +2 day readings, the velella materials can be ranked in ascending order of their phlogistic capacity: deproteinated, deacetylated, and whole velella float material (see Figure 10). Like carrageenin, when the velella materials were suspended in 0.9% saline and injected, there was no evidence of the swelling or of secondary lesions in the contralateral paws characteristically associated with the onset of Mycobacterium butyricum adjuvant polyarthritis (see Table IV and Figures 11-13).

The results shown in Tables V and VI and in Figures 14-15 are from a study which was performed in order to attempt to reproduce the results which were obtained in the last study. Since deproteinated velella float material did not seem to possess significant phlogistic capacity, it was elimated from this study. Readings were also conducted at certain time intervals not covered in the preceeding study (-5 min. and +5 hr.). Whole velella float material and partly deacetylated velella float material, like carrageenin, were suspended in 0.9% saline and studied at levels identical (mg. for mg.) to those used previously.

Carrageenin produced a rapid-onset, short-term inflammation in the injected paw which appeared at +1 hr., reached a peak at +3 hr., and which disappeared after +2 days. Through +1 day, this swelling was statistically significantly different from the saline vehicle control at a probability level of $p < 0.001$. This information correlates well with the results obtained in the previous study

Table V: Mean injected paw volumes of rats with carrageenin-induced inflammation or velella-induced inflammation (phlogiston vehicle: 0.9% saline) -- confirmation study

	N	Preinj. volume, ml.	Change from preinjection volume, ml. (± 1 SEM)						
			+5 min.	+1 hr.	+3 hr.	+5 hr.	+1 d.	+2 d.	+3 d.
0.9% Saline vehicle control	10	1.20	+0.13 (0.01)	+0.09 (0.02)	+0.08 (0.02)	+0.11 (0.02)	+0.01 (0.03)	+0.03 (0.02)	+0.00 (0.02)
Carrageenin	10	1.18	+0.17 (0.03)	+0.51[c] (0.05)	+0.77[c] (0.05)	+0.60[c] (0.07)	+0.21[c] (0.02)	+0.13[b] (0.02)	+0.02 (0.04)
Whole velella float material	10	1.23	+0.09 (0.02)	+0.11 (0.04)	+0.45[c] (0.06)	+0.43[c] (0.04)	+0.27[c] (0.03)	+0.12[a] (0.02)	+0.12[b] (0.03)
Partly deacetylated velella float material	9	1.12	+0.10 (0.03)	+0.09 (0.03)	+0.29[b] (0.06)	+0.20 (0.05)	+0.15[a] (0.05)	+0.13[b] (0.02)	+0.13[b] (0.03)

Table V continued.

	Change from preinjection volume, ml. (±1 SEM)								
	+4 d.	+5 d.	+6 d.	+7 d.	+8 d.	+9 d.	+10 d.	+11 d.	+12 d.
0.9% Saline vehicle control	+0.01 (0.02)	-0.01 (0.04)	+0.05 (0.04)	+0.04 (0.03)	+0.03 (0.02)	-0.01 (0.03)	-0.04 (0.04)	-0.04 (0.02)	+0.01 (0.04)
Carrageenin	+0.04 (0.02)	+0.07 (0.02)	+0.11 (0.02)	+0.06 (0.02)	+0.07[a] (0.03)	+0.02 (0.02)	+0.02 (0.03)	-0.02 (0.03)	-0.03 (0.06)
Whole velella float material	+0.16[b] (0.04)	+0.15[b] (0.03)	+0.22[b] (0.03)	+0.16[a] (0.04)	+0.13[a] (0.04)	+0.07 (0.03)	+0.02 (0.03)	+0.03 (0.05)	-0.01 (0.04)
Partly deacetylated velella float material	+0.15[b] (0.03)	+0.16[a] (0.04)	+0.21[b] (0.02)	+0.23[b] (0.02)	+0.15[b] (0.03)	+0.10[a] (0.03)	+0.07[a] (0.03)	+0.02 (0.03)	+0.07 (0.02)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Table VI: Mean contralateral paw volumes of rats with carrageenin-induced inflammation or velella-induced inflammation (phlogiston vehicle: 0.9% saline) -- confirmation study

	N	Preinj. volume, ml.	Change from preinjection volume, ml. (±1 SEM)						
			+5 min.	+1 hr.	+3 hr.	+5 hr.	+1 d.	+2 d.	+3 d.
0.9% Saline vehicle control	10	1.21	-0.03 (0.03)	-0.01 (0.02)	-0.01 (0.02)	+0.01 (0.02)	+0.00 (0.03)	+0.01 (0.04)	-0.01 (0.03)
Carrageenin	10	1.22	-0.02 (0.02)	-0.03 (0.01)	-0.01 (0.02)	-0.05 (0.03)	-0.06[a] (0.02)	-0.02 (0.01)	-0.05 (0.04)
Whole velella float material	10	1.24	-0.05 (0.01)	-0.03 (0.03)	-0.05 (0.01)	-0.10 (0.05)	-0.03 (0.03)	-0.02 (0.02)	-0.05 (0.04)
Partly deacetylated velella float material	9	1.10	-0.04 (0.02)	-0.01 (0.04)	+0.02 (0.04)	-0.03 (0.05)	-0.01 (0.03)	+0.01 (0.04)	+0.00 (0.03)

Table VI continued.

Change from preinjection volume, ml. (±1 SEM)								
+4 d.	+5 d.	+6 d.	+7 d.	+8 d.	+9 d.	+10 d.	+11 d.	+12 d.
+0.01 (0.03)	-0.02 (0.04)	+0.09 (0.04)	+0.06 (0.03)	+0.03 (0.02)	+0.04 (0.03)	-0.02 (0.05)	+0.01 (0.02)	+0.02 (0.04)
-0.05 (0.02)	-0.01 (0.02)	+0.03 (0.02)	-0.01 (0.02)	+0.03 (0.03)	-0.02 (0.03)	-0.02 (0.03)	-0.05 (0.04)	-0.04 (0.05)
-0.01 (0.04)	+0.01 (0.03)	+0.06 (0.02)	+0.06 (0.04)	+0.07 (0.03)	-0.04 (0.03)	+0.01 (0.03)	-0.05 (0.03)	-0.05 (0.03)
-0.01 (0.03)	+0.03 (0.04)	+0.10 (0.04)	+0.09 (0.03)	+0.10 (0.03)	+0.06 (0.03)	+0.04 (0.03)	+0.01 (0.03)	+0.08 (0.06)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

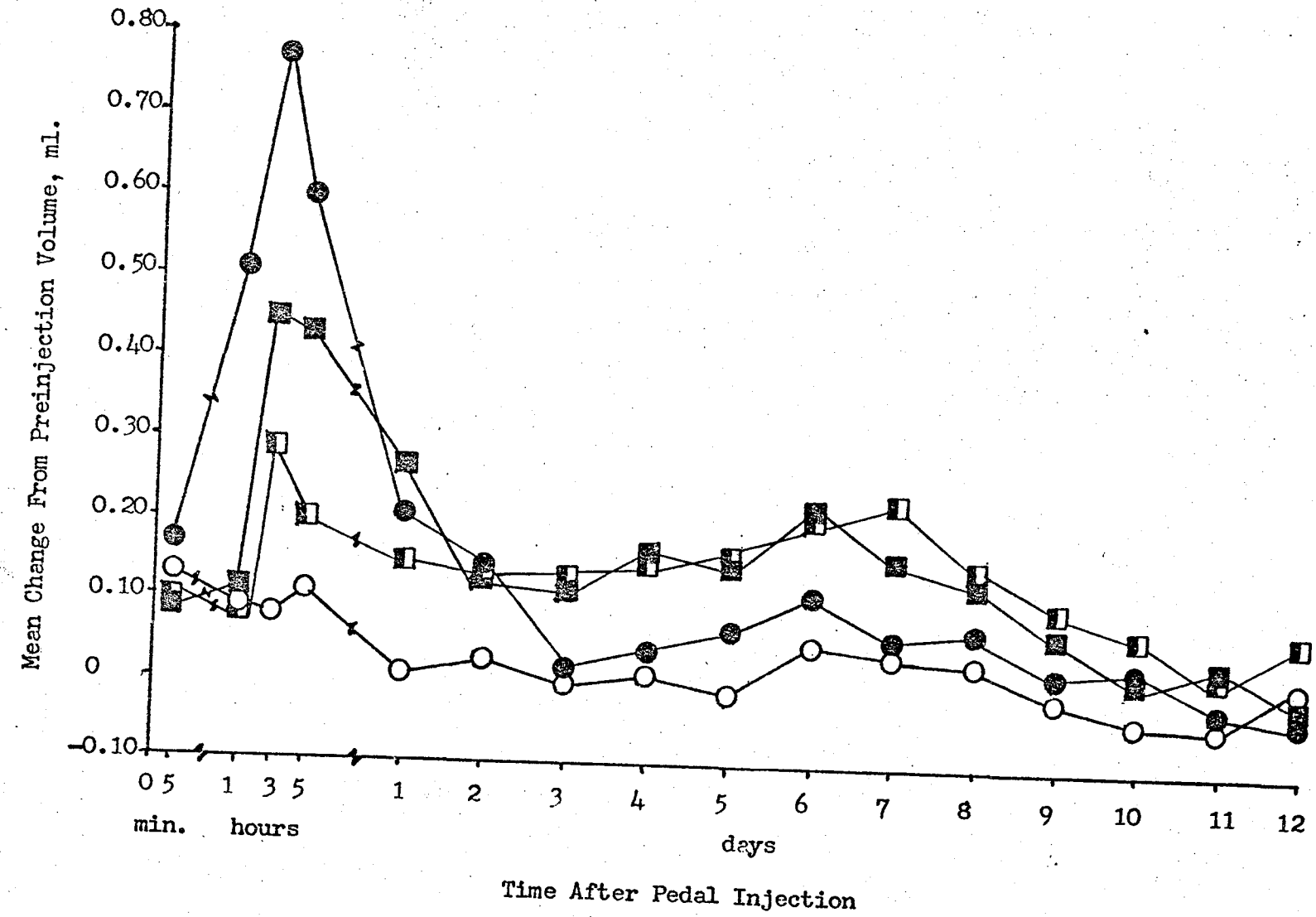

0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
Mean Change From Preinjection Volume, ml.
0 5
1 3 5
1 2 3 4 5 6 7 8 9 10 11 12
min.
hours
days
Time After Pedal Injection

Fig. 15 -- A comparison of the types of inflammation induced in the contralateral paws of animals by carrageenin, whole velella float material, and partly deacetylated velella float material.
(phlogiston vehicle: 0.9% saline)

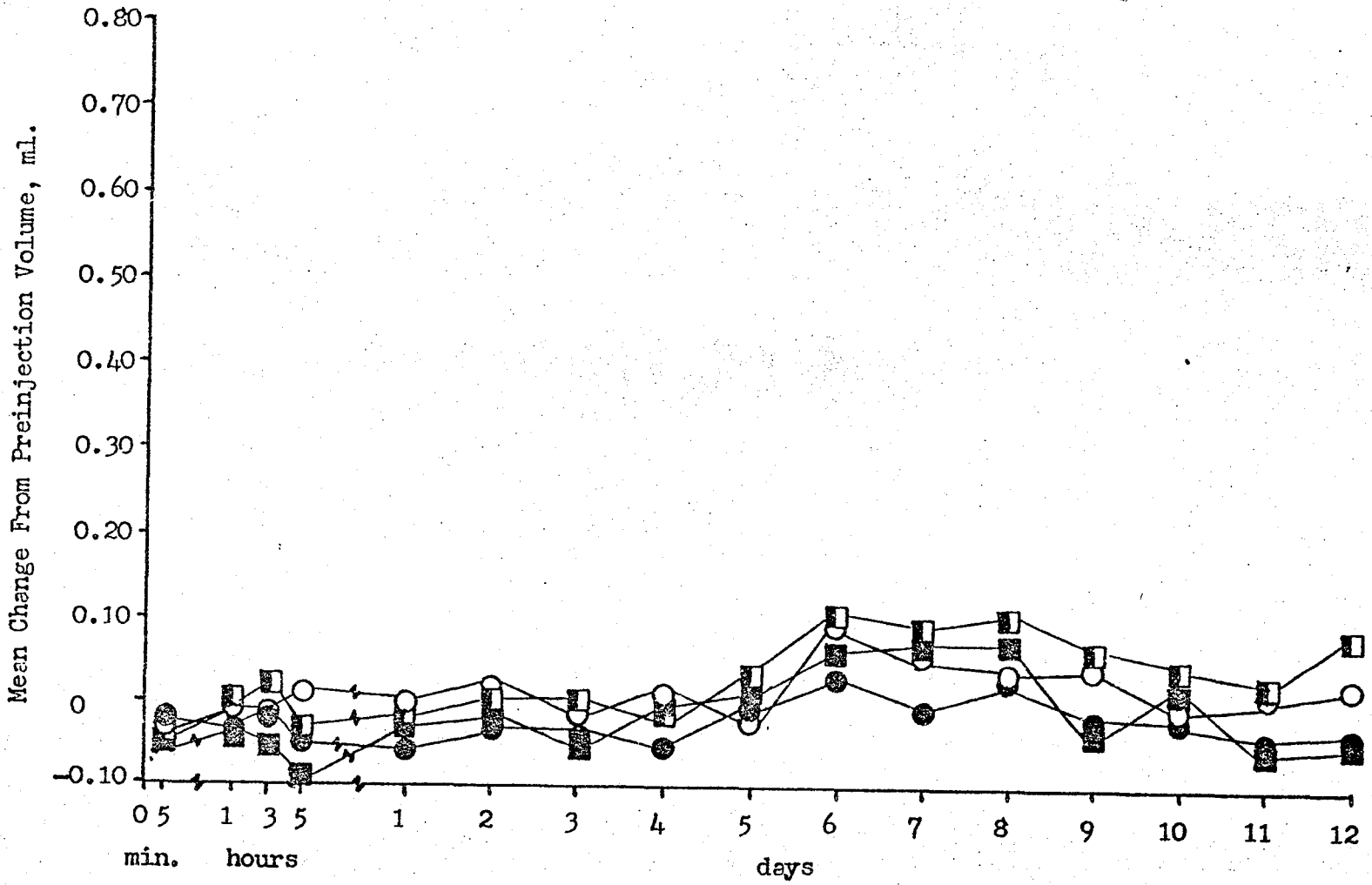
Values indicate change from preinjection volume, ml.

● carrageenin

■ whole velella

◧ deacetylated velella

○ 0.9% saline


0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
Mean Change From Preinjection Volume, ml.
0 5 1 3 5 1 2 3 4 5 6 7 8 9 10 11 12
min. hours
days
Time After Pedal Injection

(see Table V vs. Table III). Whole velella float material and partly deacetylated velella float material also produced a rapid-onset, carrageenin-like inflammation and, again, this inflammation was not as intense. Whole velella float material produced an inflammation two-thirds as intense as that produced by carrageenin. In this study, whole velella float material produced a peak swelling of 0.45 ml. at +3 hrs (see Table V and Figure 14), whereas, in the last study, this material produced a peak swelling of 0.51 ml (see Table III). Partly deacetylated velella material produced an inflammation which was only about one-third as great as that produced by carrageenin. In this study, deacetylated velella float material produced a peak swelling of o.29 ml. at +3 hrs (see Table V and Figure 14), while in the previous study, deacetylated material produced a peak swelling of 0.36 ml. (see Table III). The velella materials, in both studies, produced a type of inflammation which persisted much longer than the inflammation produced by carrageenin (see Tables III and V, and Figures 7-10 and 14). On an equal weight basis, whole velella float material appeared to possess more phlogistic ability than the deacetylated velella material (see Table V and Figure 14). This information is consistent with the information gathered in the previous study. Like carrageenin, there was absolutely no evidence of swelling or of secondary lesions in the contralateral paws of animals receiving injections of vella materials (see Table Vi and Figure 15).

The results shown in Tables VII and VIII, and in Figures 16-19 are from a study performed in order not only to reproduce the data we had already gathered about the phlogistic capacities of whole and deacetylated velella float materials, but also, and perhaps

Table VII: Mean injected paw volumes of rats with pedal edema induced by carrageenin or cotton as contrasted with pedal edema induced by certain chitinous materials (phlogiston vehicle: 0.9% saline)

	N	Preinj. volume, ml.	Change from preinjection volume, ml. (1 SEM)						
			+5 min.	+1 hr.	+3 hr.	+5 hr.	+1 d.	+3 d.	+6 d.
0.9% saline vehicle control	9	1.28	+0.19 (0.03)	+0.10 (0.03)	+0.14 (0.02)	+0.11 (0.03)	+0.02 (0.03)	-0.03 (0.02)	-0.10 (0.04)
Carrageenin	10	1.26	+0.12 (0.03)	+0.46[c] (0.05)	+0.94[c] (0.06)	+0.89[c] (0.08)	+0.24[c] (0.03)	+0.05[a] (0.03)	-0.05 (0.03)
Cotton	10	1.24	+0.16 (0.03)	+0.10 (0.02)	+0.20 (0.04)	+0.18 (0.03)	+0.11[a] (0.03)	+0.07 (0.05)	+0.01[a] (0.03)
Whole velella float material	10	1.21	+0.14 (0.02)	+0.14 (0.03)	+0.40[c] (0.05)	+0.40[c] (0.03)	+0.20[c] (0.02)	+0.11[b] (0.04)	+0.04[a] (0.03)
Partly deacetylated velella float material	10	1.25	+0.15 (0.04)	+0.18 (0.03)	+0.33[b] (0.05)	+0.22 (0.05)	+0.13 (0.05)	+0.08[a] (0.04)	+0.02 (0.05)
Partly deacetylated shrimp material	10	1.24	+0.07[a] (0.03)	+0.05 (0.04)	+0.34[b] (0.05)	+0.32[b] (0.04)	+0.08 (0.05)	+0.06[a] (0.03)	-0.04 (0.04)
Partly deacetylated loligo pen material	10	1.23	+0.19 (0.04)	+0.09 (0.03)	+0.43[b] (0.07)	+0.58[c] (0.07)	+0.30[c] (0.04)	+0.28[c] (0.06)	+0.21[c] (0.06)

Table VII continued.

	Change from preinjection volume, ml. (1 SEM)				
	+9 d.	+12 d.	+15 d.	+18 d.	+21 d.
0.9% saline vehicle control	-0.04 (0.03)	-0.05 (0.03)	-0.03 (0.03)	-0.01 (0.04)	-0.04 (0.02)
Carrageenin	+0.01 (0.03)	-0.03 (0.03)	-0.01 (0.02)	-0.07 (0.03)	-0.02 (0.02)
Cotton	+0.04[a] (0.02)	+0.08[b] (0.02)	-0.01 (0.04)	+0.01 (0.02)	-0.02 (0.03)
Whole velella float material	+0.07 (0.04)	+0.03 (0.03)	+0.11[b] (0.04)	+0.09[a] (0.03)	+0.05 (0.05)
Partly deacetylated velella float material	+0.01 (0.04)	+0.06[a] (0.03)	+0.02 (0.04)	+0.03 (0.04)	-0.01 (0.04)
Partly deacetylated shrimp material	-0.04 (0.03)	-0.01 (0.02)	-0.02 (0.04)	-0.02 (0.02)	-0.02 (0.03)
Partly deacetylated loligo pen material	+0.23[c] (0.05)	+.029[c] (0.05)	+0.23[c] (0.04)	+0.25[b] (0.06)	+0.24[b] (0.07)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Table VIII: Mean contralateral paw volumes of rats with pedal edema induced by carrageenin or cotton as contrasted with pedal edema induced by certain chitinous materials (phlogiston vehicle: 0.9% saline)

	N	Preinj. volume, ml.	Change from preinjection volume, ml. (± 1SEM)						
			+5 min.	+1 hr.	+3 hr.	+5 hr.	+1 d.	+3 d.	+6 d.
0.9% saline vehicle control	9	1.32	-0.02 (0.04)	-0.07 (0.03)	-0.07 (0.03)	-0.02 (0.05)	-0.05 (0.03)	-0.06 (0.03)	-0.12 (0.06)
Carrageenin	10	1.28	-0.01 (0.02)	-0.04 (0.03)	+0.03 (0.06)	-0.02 (0.03)	-0.06 (0.02)	-0.07 (0.03)	-0.07 (0.04)
Cotton	10	1.24	-0.04 (0.03)	+0.03[a] (0.02)	+0.03[a] (0.02)	-0.04 (0.02)	-0.05 (0.02)	-0.01 (0.02)	-0.08 (0.03)
Whole velella float material	10	1.24	+0.00 (0.03)	-0.02 (0.03)	+0.02[a] (0.02)	-0.01 (0.02)	-0.03 (0.03)	-0.02 (0.03)	-0.04 (0.04)
Partly deacetylated velella float material	10	1.26	+0.02 (0.03)	-0.01 (0.02)	+0.02[a] (0.03)	-0.05 (0.02)	-0.04 (0.02)	-0.04 (0.02)	-0.08 (0.03)
Partly deacetylated shrimp material	10	1.24	+0.01 (0.02)	-0.07 (0.03)	-0.02 (0.03)	-0.04 (0.03)	-0.12 (0.03)	-0.01 (0.04)	-0.08 (0.05)
Partly deacetylated loligo pen material	10	1.21	+0.05 (0.03)	-0.01 (0.03)	+0.06[b] (0.02)	+0.04 (0.03)	-0.03 (0.03)	+0.07 (0.05)	+0.02 (0.05)

Table VIII continued.

	Change from preinjection volume, ml. (1 SEM)				
	+9 d.	+12 d.	+15 d.	+18 d.	+21 d.
0.9% saline vehicle control	-0.08 (0.03)	-0.06 (0.04)	-0.07 (0.03)	-0.09 (0.05)	-0.08 (0.03)
Carrageenin	-0.07 (0.03)	-0.08 (0.03)	-0.09 (0.03)	-0.10 (0.03)	-0.09 (0.03)
Cotton	-0.03 (0.02)	-0.02 (0.02)	-0.05 (0.02)	-0.06 (0.01)	-0.07 (0.03)
Whole velella float material	-0.06 (0.04)	-0.02 (0.02)	-0.04 (0.02)	-0.08 (0.03)	-0.08 (0.02)
Partly deacetylated velella float material	-0.06 (0.03)	-0.03 (0.05)	-0.06 (0.03)	-0.05 (0.02)	-0.09 (0.02)
Partly deacetylated shrimp material	-0.07 (0.02)	-0.07 (0.01)	-0.09 (0.02)	-0.07 (0.03)	-0.13 (0.03)
Partly deacetylated loligo pen material	+0.03[a] (0.02)	+0.08[b] (0.02)	+0.04[a] (0.03)	+0.02[a] (0.02)	+0.00[a] (0.02)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Fig. 16 - A comparison of the types of inflammation induced in the injected paws of animals by carrageenin, whole velella, partly deacetylated velella, and cotton.

(phlogiston vehicle: 0.9% saline)

Values indicate change from preinjection volume, ml.

- carrageenin
- whole velella
- deacetylated velella
- cotton
- 0.9% saline

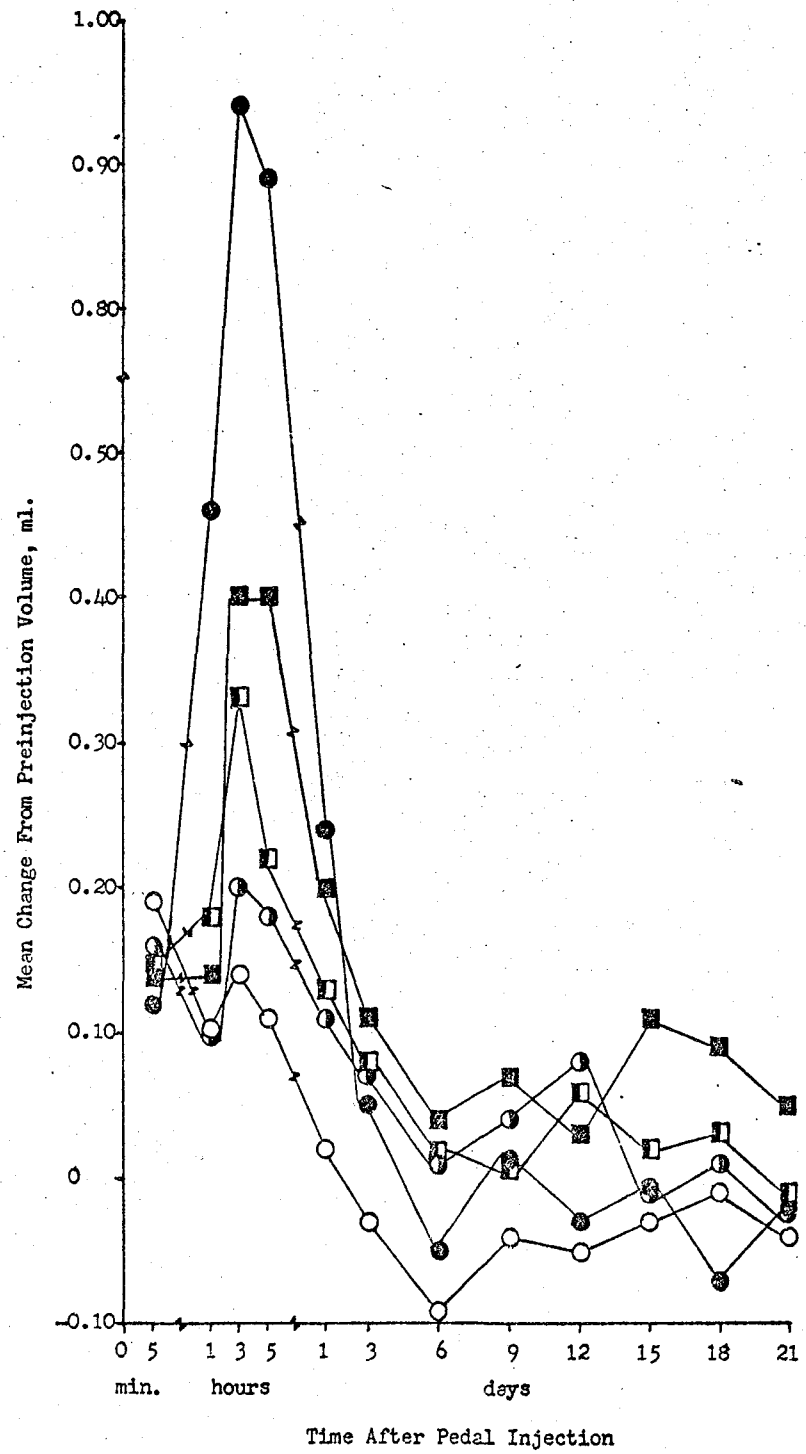

1.00
0.90
0.80
0.50
0.40
0.30
0.20
0.10
0
-0.10
Mean Change From Preinjection Volume, ml.
0 5 1 3 5 1 3 6 9 12 15 18 21
min. hours days
Time After Pedal Injection

Fig. 17 -- A comparison of the types of inflammation induced in the injected paws of animals by deacetylated loligo, deacetylated shrimp, deacetylated velella, and cotton. (phlogiston vehicle: 0.9% saline)

Values indicate change from preinjection volume, ml.

- deacetylated loligo
- deacetylated shrimp
- deacetylated velella
- cotton
- 0.9% saline

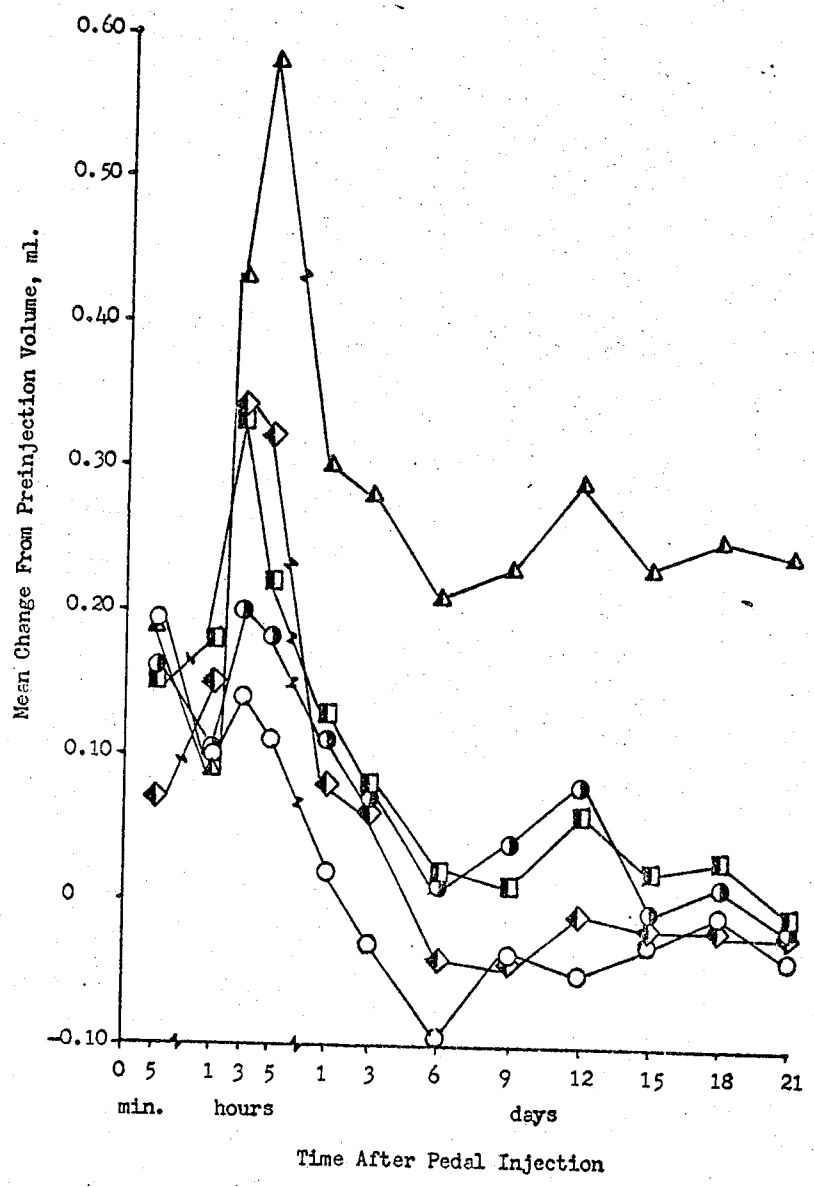

0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
Mean Change From Preinjection Volume, ml.
0 5 1 3 5 1 3 6 9 12 15 18 21
min. hours days
Time After Pedal Injection

Fig. 18 -- A comparison of the types of inflammation induced in the contralateral paws of animals by carrageenin, whole velella, partly deacetylated velella, and cotton.

(phlogiston vehicle: 0.9% saline)

Values indicate change from preinjection volume, ml.

- carrageenin
- whole velella
- deacetylated velella
- cotton
- 0.9% saline

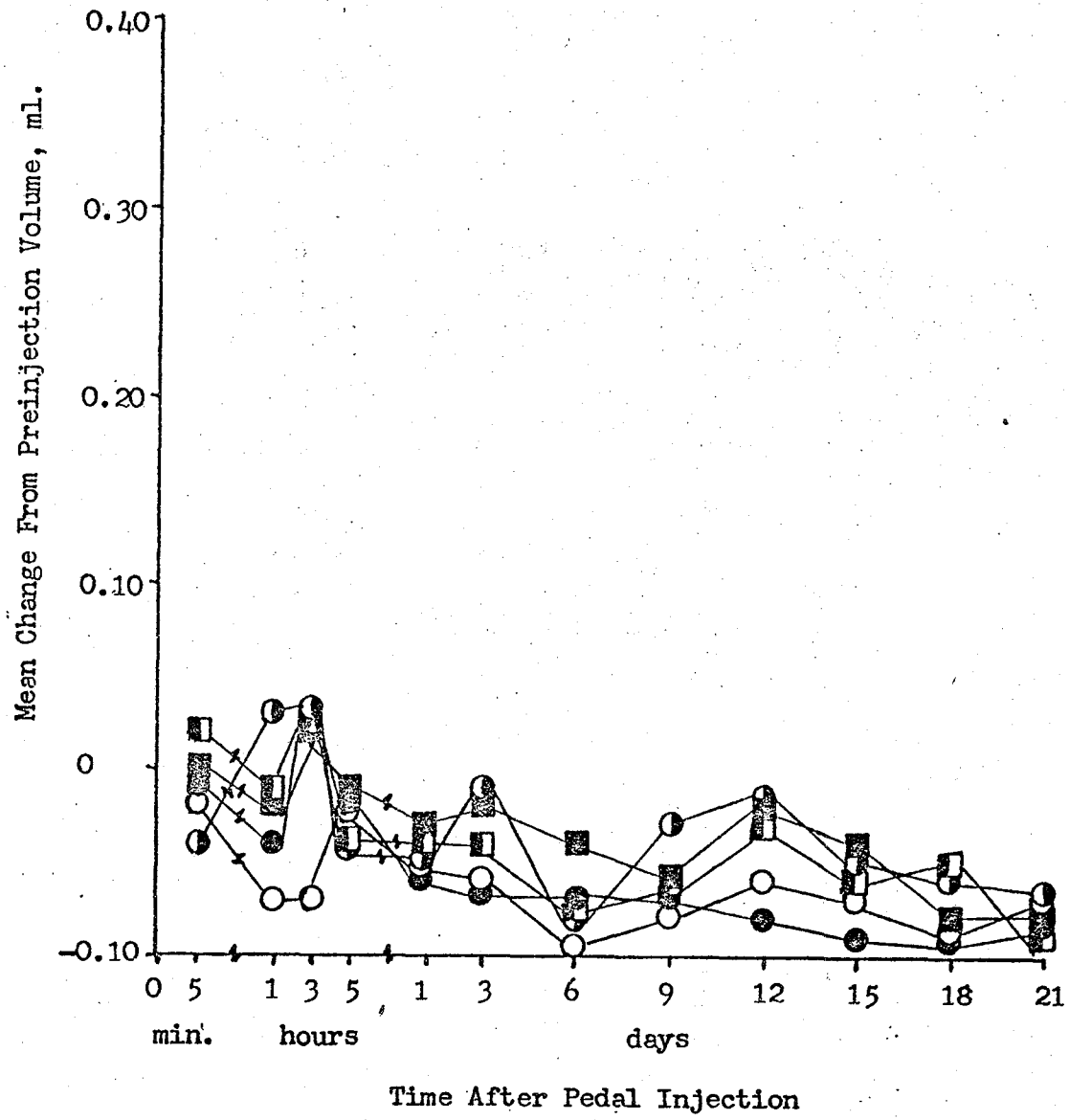
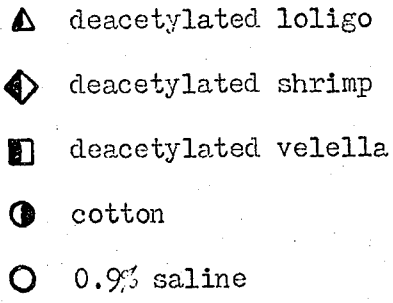
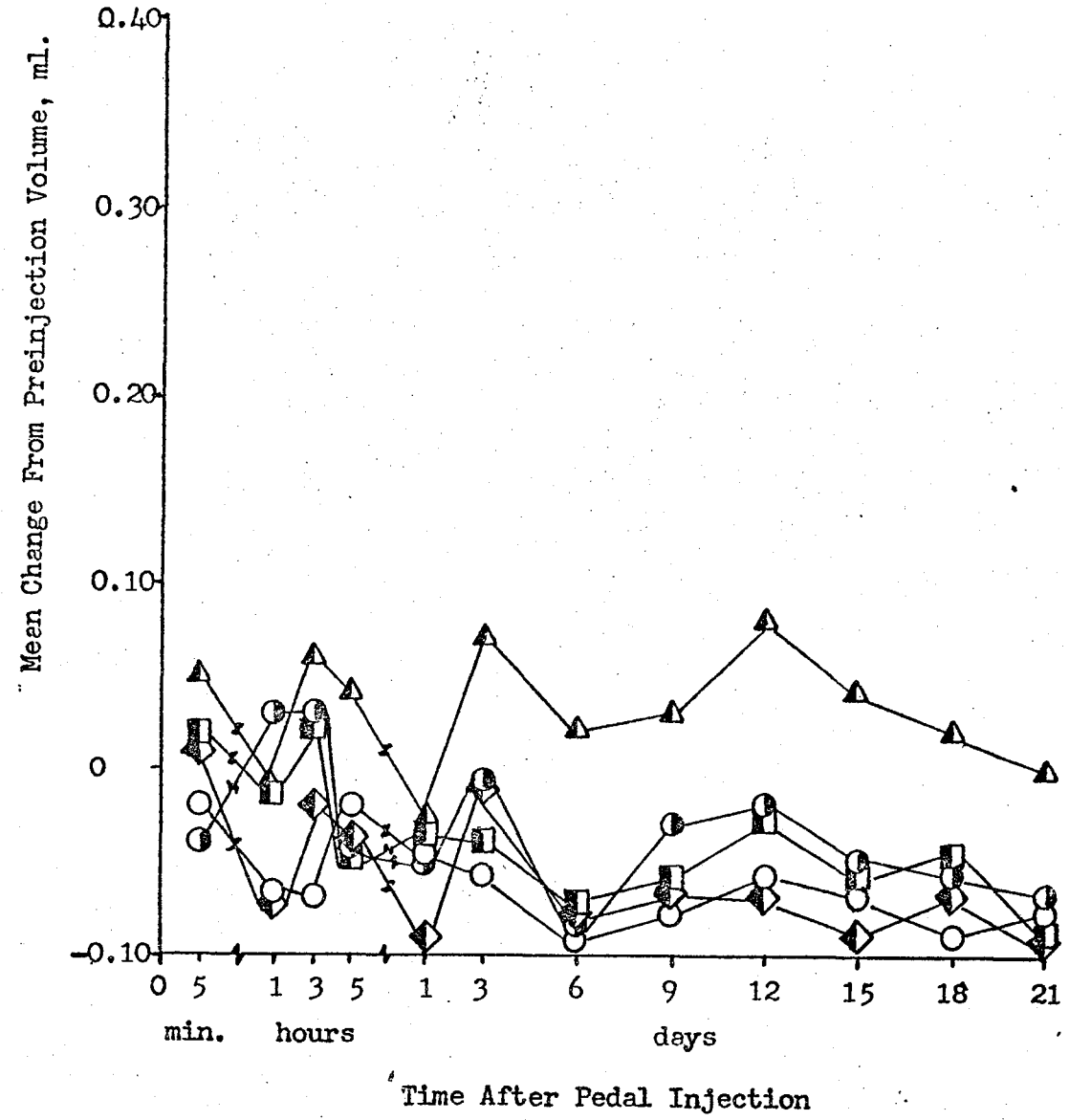

0.40
0.30
0.20
0.10
0
-0.10
Mean Change From Preinjection Volume, ml.
0 5 1 3 5 1 3 6 9 12 15 18 21
min. hours days
Time After Pedal Injection

Fig. 19 -- A comparison of the types of inflammation induced in the contralateral paws of animals by deacetylated loligo, deacetylated shrimp, deacetylated velella, and cotton.

(phlogiston vehicle: 0.9% saline)

Values indicate change from preinjection volume, ml.




Mean Change From Preinjection Volume, ml.
0.40
0.30
0.20
0.10
0
-0.10
0 5
1 3 5
1 3
6
9
12
15
18
21
min.
hours
days
Time After Pedal Injection

more importantly, to demonstrate the phlogistic relationship of these materials to other chitinous materials. All materials were suspended in 0.9% saline and appropriate controls were utilized. The phlogistic capacity of cotton was also analyzed in this study.

As expected, carrageenin produced a rapid-onset, short-term inflammation in the injected paw which appeared at +1 hr., reached a peak of 0.94 ml. at +3 hrs., and which then disappeared after +3 days. The probability level of significance for carrageenin inflammation was $p < 0.001$ up through +1 day (See Table VII). Whole velella float material and partly deacetylated velella float material also produced a rapid-onset type inflammation appearing and becoming statistically significant at +3 hrs., but this inflammation was not as intense as that produced by carrageenin. Whole velella float material produced a peak in swelling of +0.40 ml. at +3 - 5 hrs. and the probability level of significance was $p < 0.001$. This amount of swelling was only about one-third as intense as that produced by carrageenin. All this information is consistent with the information reported in the two previous studies. Partly deacetylated shrimp material and partly deacetylated loligo pen material also produced a rapid-onset type of inflammation, reaching peak effect at +3 hr. and +5 hr. respectively. Partly deacetylated shrimp material, like partly deacetylated velella float material, seemed to produce an inflammation which was about one-third as intense as that produced by carrageenin. Partly deacetylated loligo pen material, on the other hand, seemed to produce an inflammation which was about two-thirds as intense as that produced by carrageenin. The inflammation produced by deacetylated shrimp had disappeared by

+3 days; however, the inflammation produced by deacetylated loligo persisted throughout the observation period (see Table VII and Figure 17). When all the deacetylated forms in this study are compared together for their phlogistic capacities, it appears that deacetylated loligo pen material possessed the most phlogistic action (peak swelling of 0.58 ml.); partly deacetylated shrimp material held the second position (peak swelling of +0.34 ml.); and partly deacetylated velella float materials showed the least phlogistic capacity (peak swelling of +0.33 ml.) (see Table VII and Figure 17). There was no evidence of swelling or of secondary lesions in the contralateral paws of animals receiving injections of any of the materials analyzed in this study (see Table VIII and Figures 18-19).

The results shown in Tables IX and X and in Figures 20-27 are from a study performed in order to determine the phlogistic capacities of the whole and deproteinated forms of loligo pen material and shrimp material. All materials were suspended in 0.9% saline and appropriate saline and carrageenin controls were run concurrently. The phlogistic capacity of cotton was also analyzed.

As in all previous studies, carrageenin produced a quick-onset, short-duration type of inflammation, producing a peak of swelling of +0.86 ml. at +3 hrs and disappearing after +2 days. Probability level of significance was $p < 0.001$ through +5 hrs. (see Table IX). Whole loligo and deproteinated loligo pen material also produced a quick-onset type of inflammation, but this inflammation was not as intense as that produced by carrageenin. Whole loligo pen material produced a peak of swelling of +0.42 ml. at +3 hrs. (about 44% of the swelling produced by carrageenin at the same time). Probability level of signi-

Table IX: Mean injected paw volumes of rats with pedal edema induced by carrageenin or cotton as contrasted with pedal edema induced by certain chitinous materials (phlogiston vehicle: 0.9% saline)

	N	Preinj. volume, ml.	Change from preinjection volume, ml. (±1SEM)						
			+5 min.	+1 hr.	+3 hr.	+5 hr.	+1 d.	+2 d.	+3 d.
0.9% saline vehicle control	10	1.18	+0.16 (0.02)	+0.16 (0.03)	+0.15 (0.03)	+0.09 (0.03)	+0.08 (0.02)	+0.07 (0.02)	+0.12 (0.03)
Carrageenin	10	1.17	+0.23 (0.06)	+0.63[c] (0.08)	+0.86[c] (0.11)	+0.86[c] (0.09)	+0.26[b] (0.05)	+0.19[b] (0.03)	+0.20 (0.04)
Cotton	10	1.17	+0.17 (0.03)	+0.20 (0.05)	+0.26 (0.06)	+0.28[b] (0.06)	+0.20[c] (0.02)	+0.13 (0.03)	+0.18 (0.01)
Whole loligo pen material	10	1.21	+0.20 (0.02)	+0.21 (0.05)	+0.42[c] (0.03)	+0.38[c] (0.05)	+0.30[b] (0.06)	+0.25[c] (0.03)	+0.29[b] (0.03)
Deproteinated loligo pen material	10	1.19	+0.18 (0.04)	+0.15 (0.04)	+0.19 (0.03)	+0.40[c] (0.06)	+0.28[b] (0.04)	+0.19[b] (0.04)	+0.20 (0.05)
Whole shrimp material	10	1.21	+0.22 (0.03)	+0.31[b] (0.04)	+0.48[c] (0.05)	+0.51[c] (0.04)	+0.29[b] (0.06)	+0.20[b] (0.03)	+0.26[b] (0.02)
Deproteinated shrimp material	10	1.17	+0.23[a] (0.02)	+0.30[a] (0.04)	+0.42[c] (0.05)	+0.40[c] (0.04)	+0.24[b] (0.04)	+0.17[b] (0.03)	+0.19 (0.03)

Table IX continued.

	Change from preinjection volume, ml. (±1 SEM)				
	+4 d.	+5 d.	+7 d.	+9 d.	+11 d.
0.9% saline vehicle control	+0.15 (0.01)	+0.20 (0.01)	+0.24 (0.03)	+0.30 (0.03)	+0.29 (0.03)
Carrageenin	+0.20 (0.03)	+0.22 (0.04)	+0.30 (0.04)	+0.29 (0.04)	+0.32 (0.04)
Cotton	+0.18 (0.01)	+0.18 (0.03)	+0.23 (0.02)	+0.28 (0.04)	+0.28 (0.05)
Whole loligo pen material	+0.24[a] (0.04)	+0.25 (0.02)	+0.29 (0.03)	+0.34 (0.02)	+0.30 (0.03)
Deproteinated loligo pen material	+0.17 (0.07)	+0.27[a] (0.03)	+0.34 (0.04)	+0.31 (0.04)	+0.30 (0.05)
Whole shrimp material	+0.30[b] (0.04)	+0.38[b] (0.05)	+0.35[a] (0.04)	+0.34 (0.05)	+0.33 (0.05)
Deproteinated shrimp material	+0.18 (0.01)	+0.19 (0.03)	+0.24 (0.02)	+0.25 (0.02)	+0.26 (0.04)

[a], [b], and [c] indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Table X: Mean contralateral paw volumes of rats with pedal edema induced by carrageenin or cotton as contrasted with pedal edema induced by certain chitinous materials (phlogiston vehicle: 0.9% saline)

	N	Preinj. volume, ml.	Change from preinjection volume, ml. (±1 SEM)						
			+5 min.	+1 hr.	+3 hr.	+5 hr.	+1 d.	+2 d.	+3 d.
0.9% saline vehicle control	10	1.18	-0.02 (0.02)	+0.00 (0.02)	-0.01 (0.01)	+0.01 (0.02)	+0.00 (0.03)	+0.04 (0.03)	+0.13 (0.03)
Carrageenin	10	1.19	+0.01 (0.03)	+0.00 (0.03)	+0.01 (0.03)	+0.01 (0.03)	+0.01 (0.04)	+0.06 (0.03)	+0.12 (0.04)
Cotton	10	1.18	+0.04 (0.03)	+0.00 (0.03)	+0.01 (0.02)	+0.02 (0.03)	+0.02 (0.04)	+0.05 (0.03)	+0.06 (0.03)
Whole loligo pen material	10	1.20	+0.02 (0.02)	+0.00 (0.02)	+0.01 (0.03)	+0.03 (0.02)	+0.02 (0.01)	+0.05 (0.02)	+0.11 (0.03)
Deproteinated loligo pen material	10	1.17	-0.03 (0.04)	-0.05 (0.04)	-0.01 (0.04)	+0.03 (0.04)	-0.02 (0.04)	+0.00 (0.03)	+0.12 (0.04)
Whole shrimp material	10	1.24	+0.05 (0.03)	+0.01 (0.03)	+0.04 (0.03)	+0.08 (0.04)	+0.00 (0.03)	+0.03 (0.02)	+0.13 (0.03)
Deproteinated shrimp material	10	1.20	+0.02 (0.02)	+0.01 (0.02)	+0.05 (0.02)	+0.05 (0.03)	+0.00 (0.03)	+0.06 (0.03)	+0.08 (0.02)

Table X continued.

	Change from preinjection volume, ml. (± 1 SEM)				
	+4 d.	+5 d.	+7 d.	+9 d.	+11 d.
0.9% saline vehicle control	+0.10 (0.02)	+0.20 (0.02)	+0.24 (0.03)	+0.23 (0.05)	+0.30 (0.03)
Carrageenin	+0.13 (0.03)	+0.20 (0.04)	+0.22 (0.02)	+0.30 (0.04)	+0.30 (0.03)
Cotton	+0.13 (0.02)	+0.16 (0.03)	+0.21 (0.02)	+0.27 (0.02)	+0.27 (0.03)
Whole loligo pen material	+0.14 (0.03)	+0.17 (0.04)	+0.28 (0.03)	+0.28 (0.04)	+0.29 (0.04)
Deproteinated loligo pen material	+0.10 (0.03)	+0.14 (0.05)	+0.18 (0.03)	+0.22 (0.04)	+0.25 (0.03)
Whole shrimp material	+0.13 (0.02)	+0.22 (0.03	+0.24 (0.03)	+0.20 (0.04)	+0.29 (0.04)
Deproteinated shrimp	+0.13 (0.02)	+0.16 (0.02)	+0.29 (0.03)	+0.23 (0.02)	+0.26 (0.04)

a, b, and c indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. For all other values, $p > 0.05$.

Fig. 20 -- A comparison of the types of inflammation induced in the injected paws of animals by carrageenin, whole loligo pen material, deproteinated loligo pen material, and cotton.
(phlogiston vehicle: 0.9% saline)

Values indicate change from preinjection volume, ml.

- ● carrageenin
- ▲ whole loligo
- ◮ deproteinated loligo
- ◑ cotton
- ○ 0.9% saline

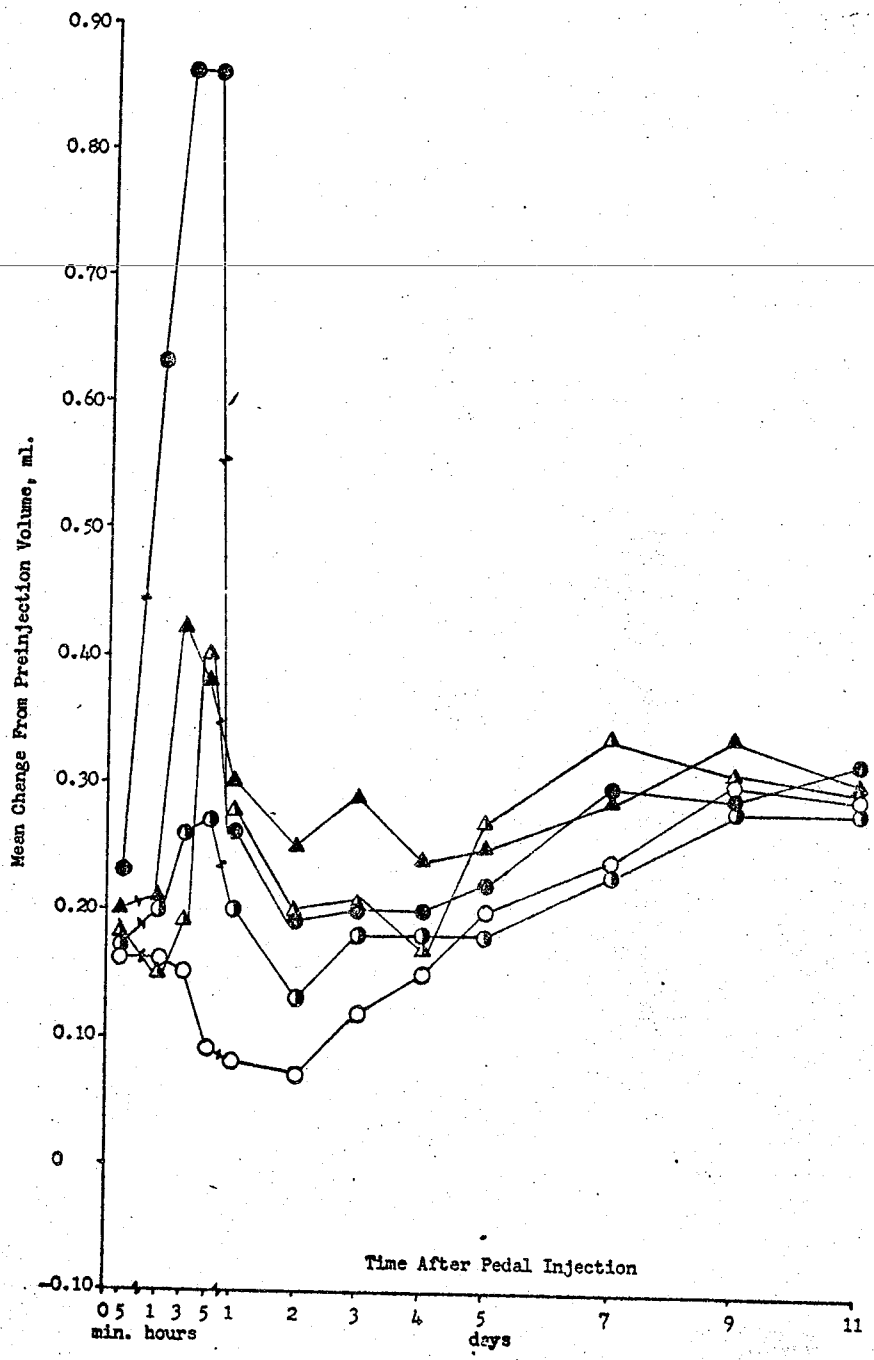

0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
Mean Change From Preinjection Volume, ml.
Time After Pedal Injection
0 5 1 3 5 1 2 3 4 5 7 9 11
min. hours
days

Fig. 21 -- A comparison of the types of inflammation induced in the injected paws of animals by carrageenin, whole shrimp material, deproteinated shrimp material, and cotton.

(phlogiston vehicle: 0.9% saline)

Values indicate change from preinjection volume, ml.

- carrageenin
- whole shrimp
- deproteinated shrimp
- cotton
- 0.9% saline

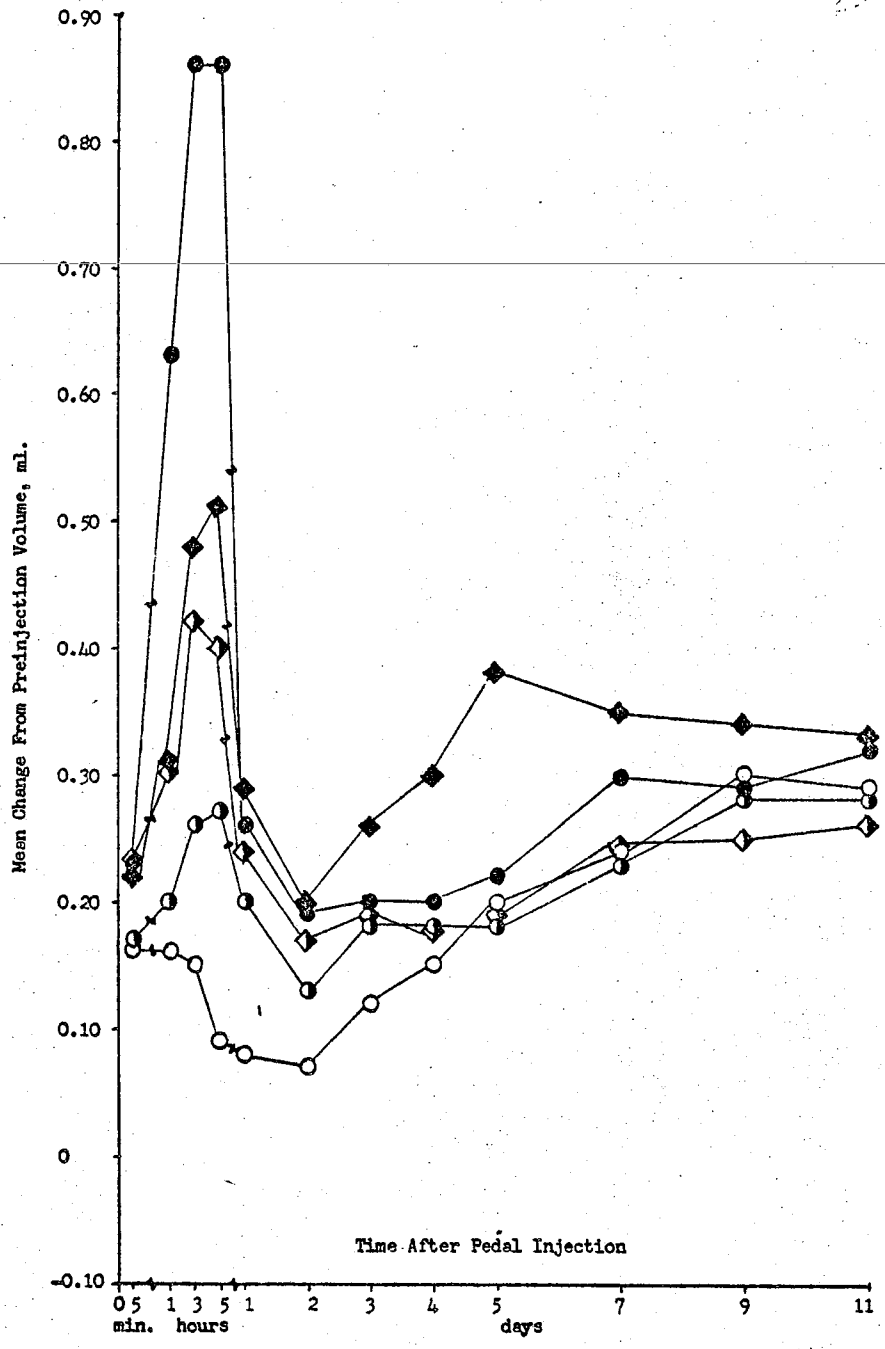

0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
Mean Change From Preinjection Volume, ml.
Time After Pedal Injection
0 5 1 3 5 1 2 3 4 5 7 9 11
min. hours days

Fig. 22 -- A comparison of the types of inflammation induced in the injected paws of animals by whole shrimp material, deproteinated shrimp material, whole loligo pen material, and deproteinated loligo pen material.
(phlogiston vehicle: 0.9% saline)

Values indicate change from preinjection volume, ml.

◆ whole shrimp
◇ deproteinated shrimp
▲ whole loligo
△ deproteinated loligo

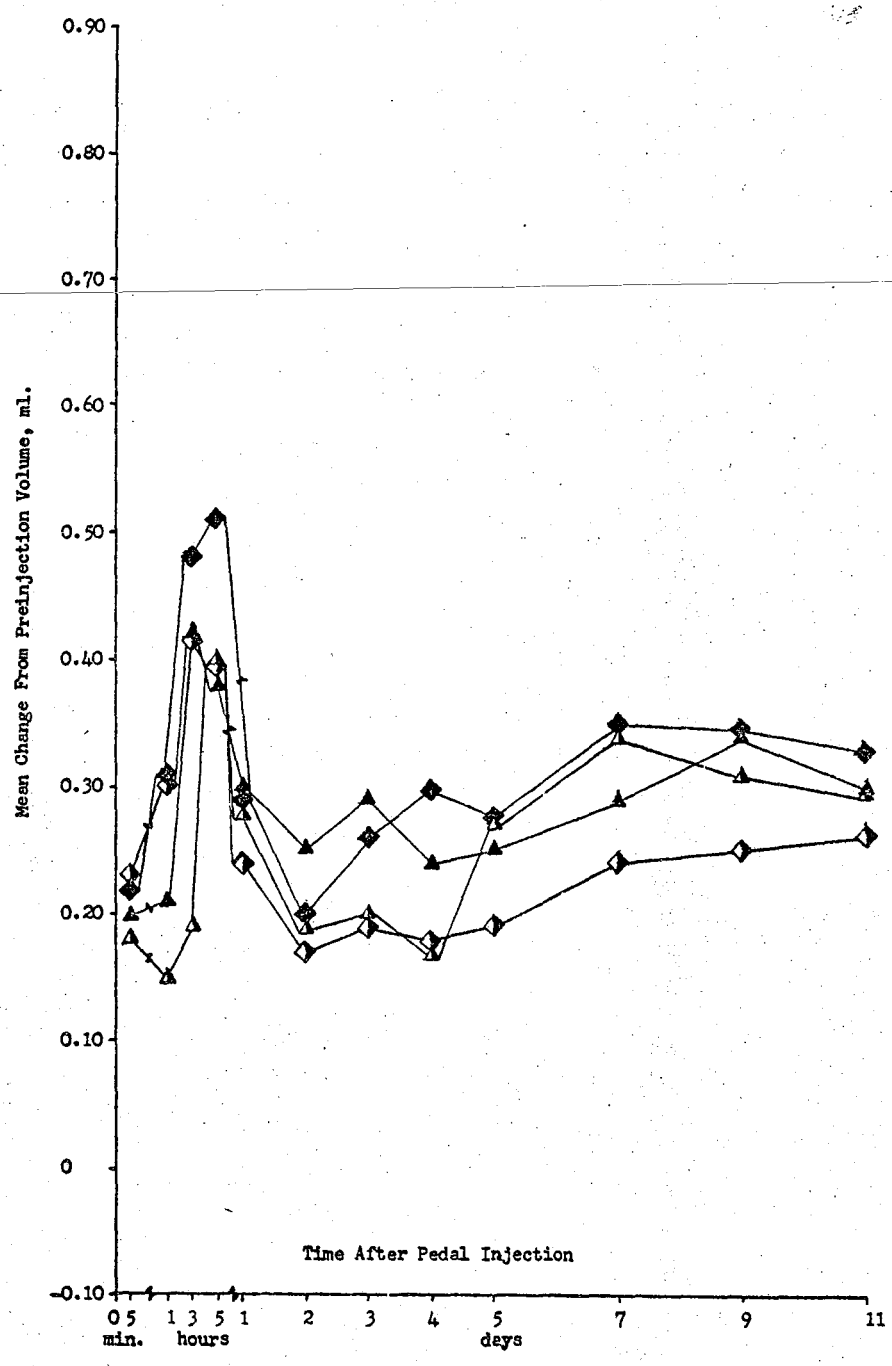

0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
Mean Change From Preinjection Volume, ml.
Time After Pedal Injection
0 5 1 3 5 1 2 3 4 5 7 9 11
min. hours days

Fig. 23 -- A comparison of the types of inflammation induced in the injected paws of animals by carrageenin, whole loligo pen material, whole shrimp material, and cotton. (phlogiston vehicle: 0.9% saline)

Values indicate change from preinjection volume, ml.

● carrageenin

▲ whole loligo

◆ whole shrimp

◐ cotton

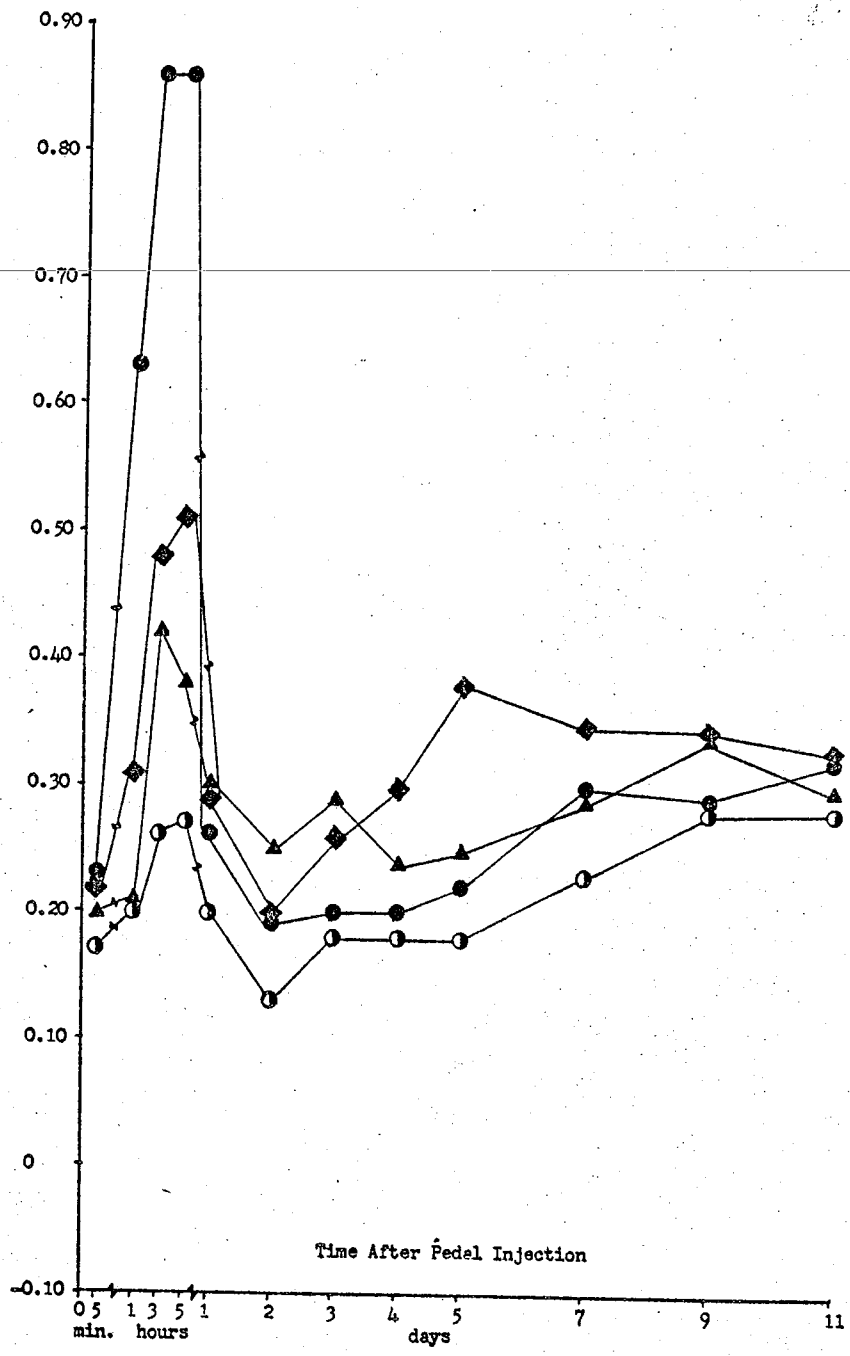
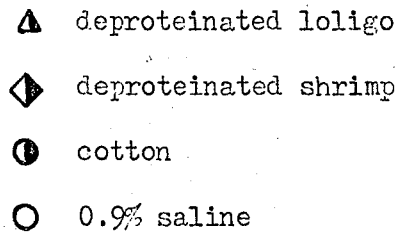

0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
Time After Pedal Injection
0 5 1 3 5 1 2 3 4 5 7 9 11
min. hours
days

Fig. 24 -- A comparison of the types of inflammation induced in the injected paws of animals by deproteinated loligo pen material, deproteinated shrimp material, and cotton.

(phlogiston vehicle: 0.9% saline)

Values indicate change from preinjection volume, ml.



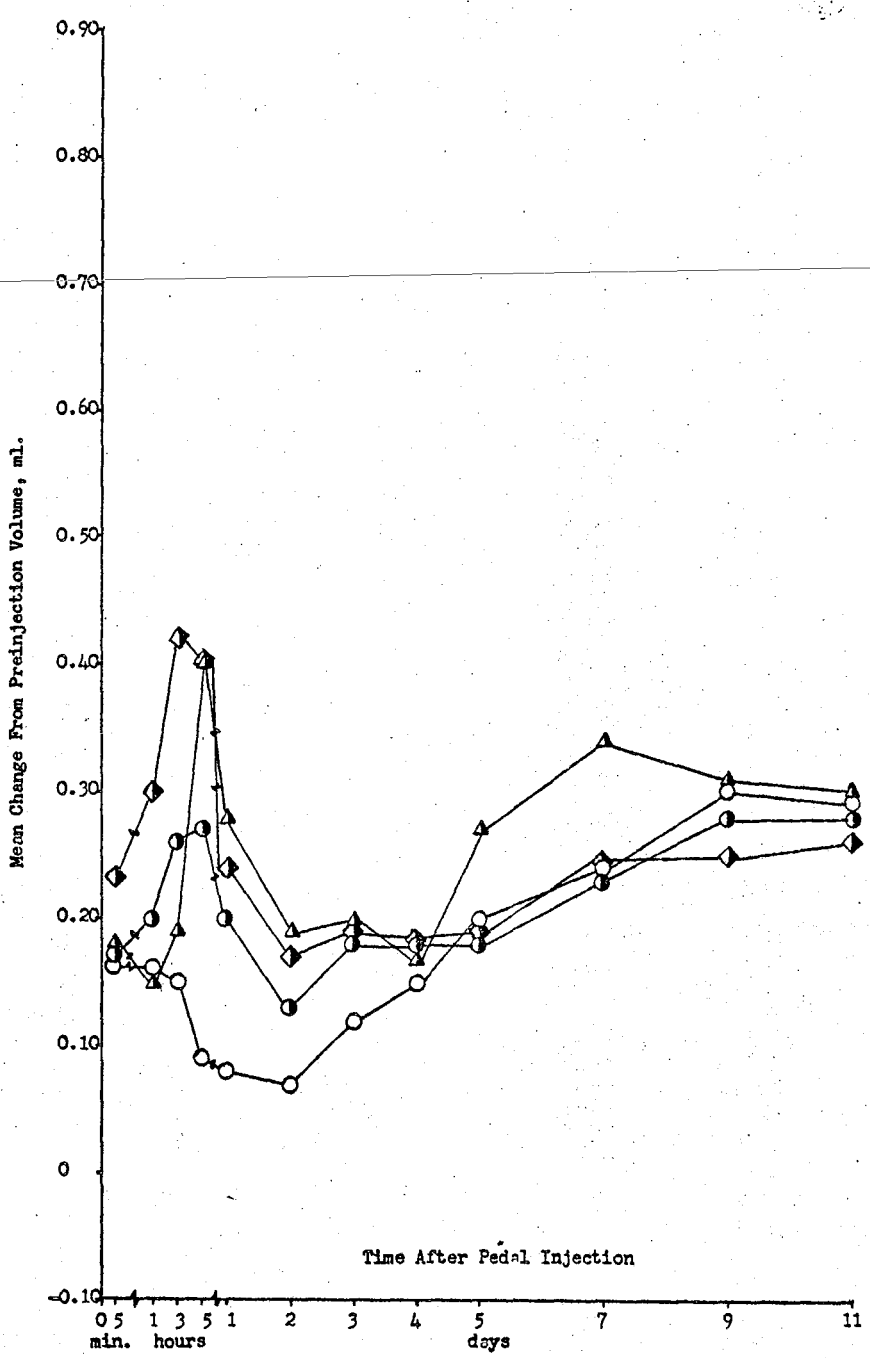

Mean Change From Preinjection Volume, ml.
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
-0.10
Time After Pedal Injection
0 5 1 3 5 1 2 3 4 5 7 9 11
min. hours days

Fig. 25 -- A comparison of the types of inflammation induced in the contralateral paws of animals by carrageenin, whole loligo pen material, deproteinated loligo pen material, and cotton.
(phlogiston vehicle: 0.9% saline)

Values indicate change from preinjection volume, ml.

● carrageenin

▲ whole loligo

◭ deproteinated loligo

◑ cotton

○ 0.9% saline

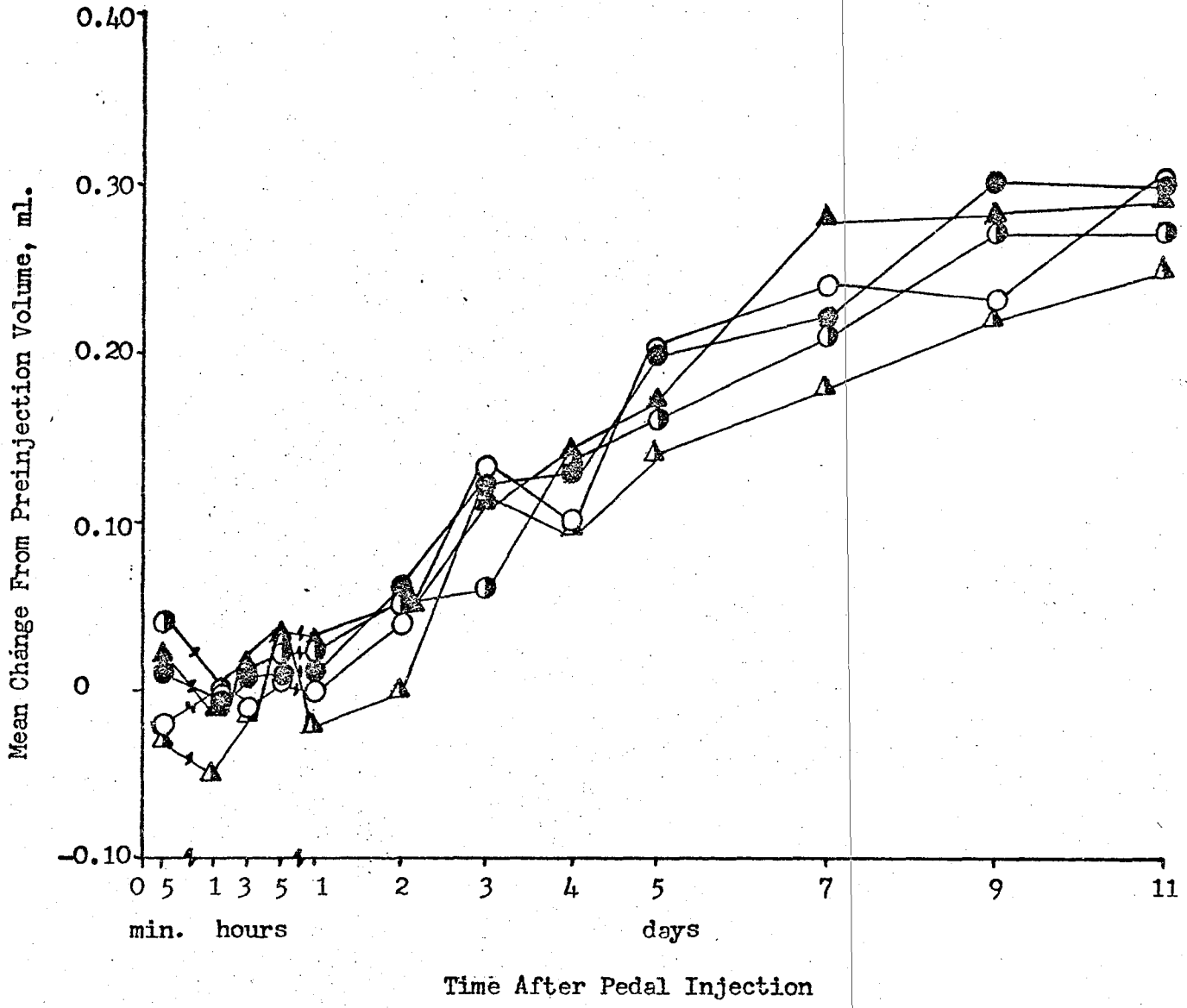

0.40
0.30
0.20
0.10
0
-0.10
Mean Change From Preinjection Volume, ml.
0 5 1 3 5 1 2 3 4 5 7 9 11
min. hours days
Time After Pedal Injection

Fig. 26 -- A comparison of the types of inflammation induced in the contralateral paws of animals by carrageenin, whole shrimp material, deproteinated shrimp material, and cotton.
(phlogiston vehicle: 0.9% saline)

Values indicate change from preinjection volume, ml.

- carrageenin
- whole shrimp
- deproteinated shrimp
- cotton
- 0.9% saline

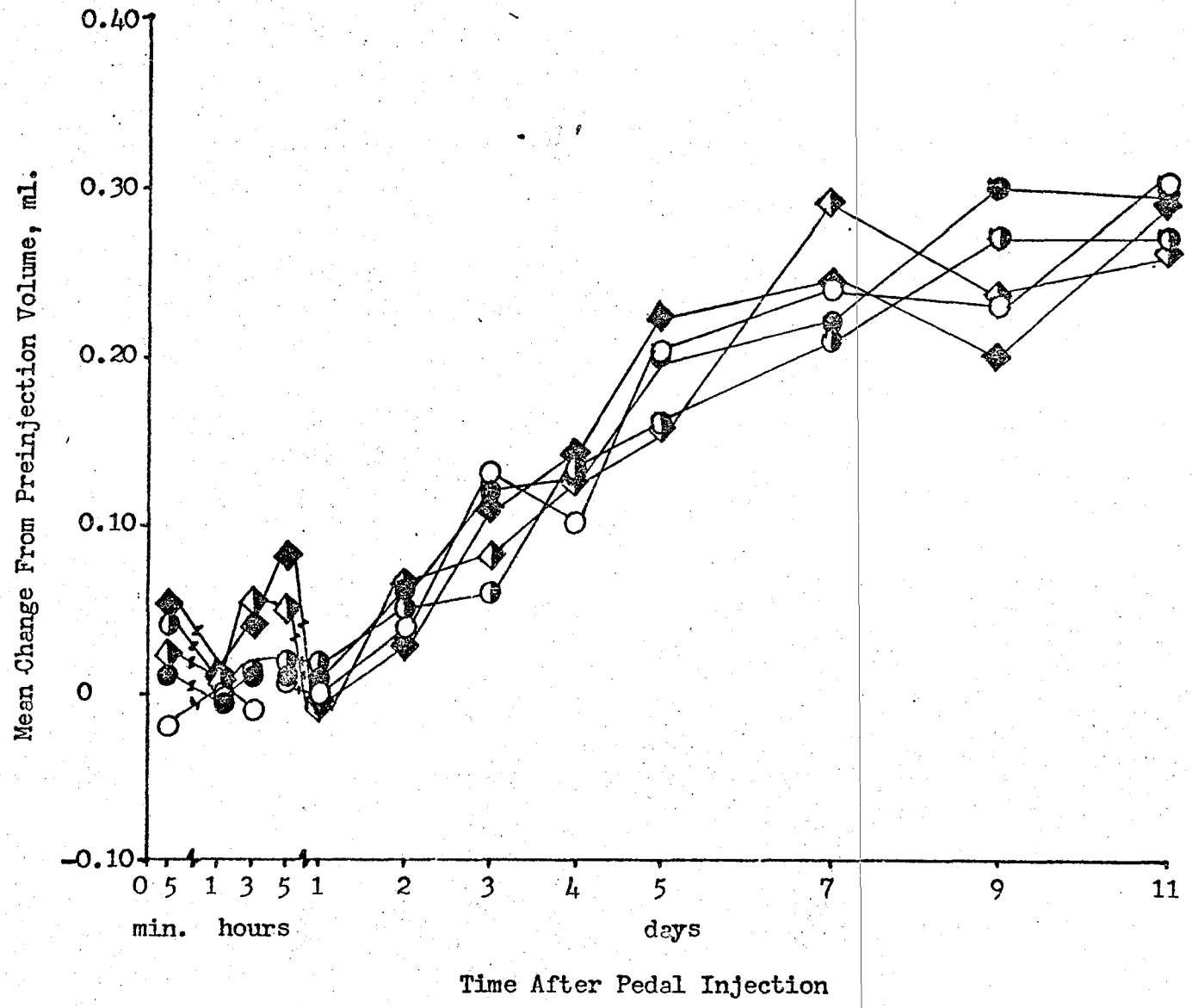

0.40
0.30
0.20
0.10
0
-0.10
Mean Change From Preinjection Volume, ml.
0 5 1 3 5 1 2 3 4 5 7 9 11
min. hours days
Time After Pedal Injection

Fig. 27 -- A comparison of the types of inflammation induced in the contralateral paws of animals by whole shrimp material, deproteinated shrimp material, whole loligo pen material, and deproteinated loligo pen material.
(phlogiston vehicle: 0.9% saline)

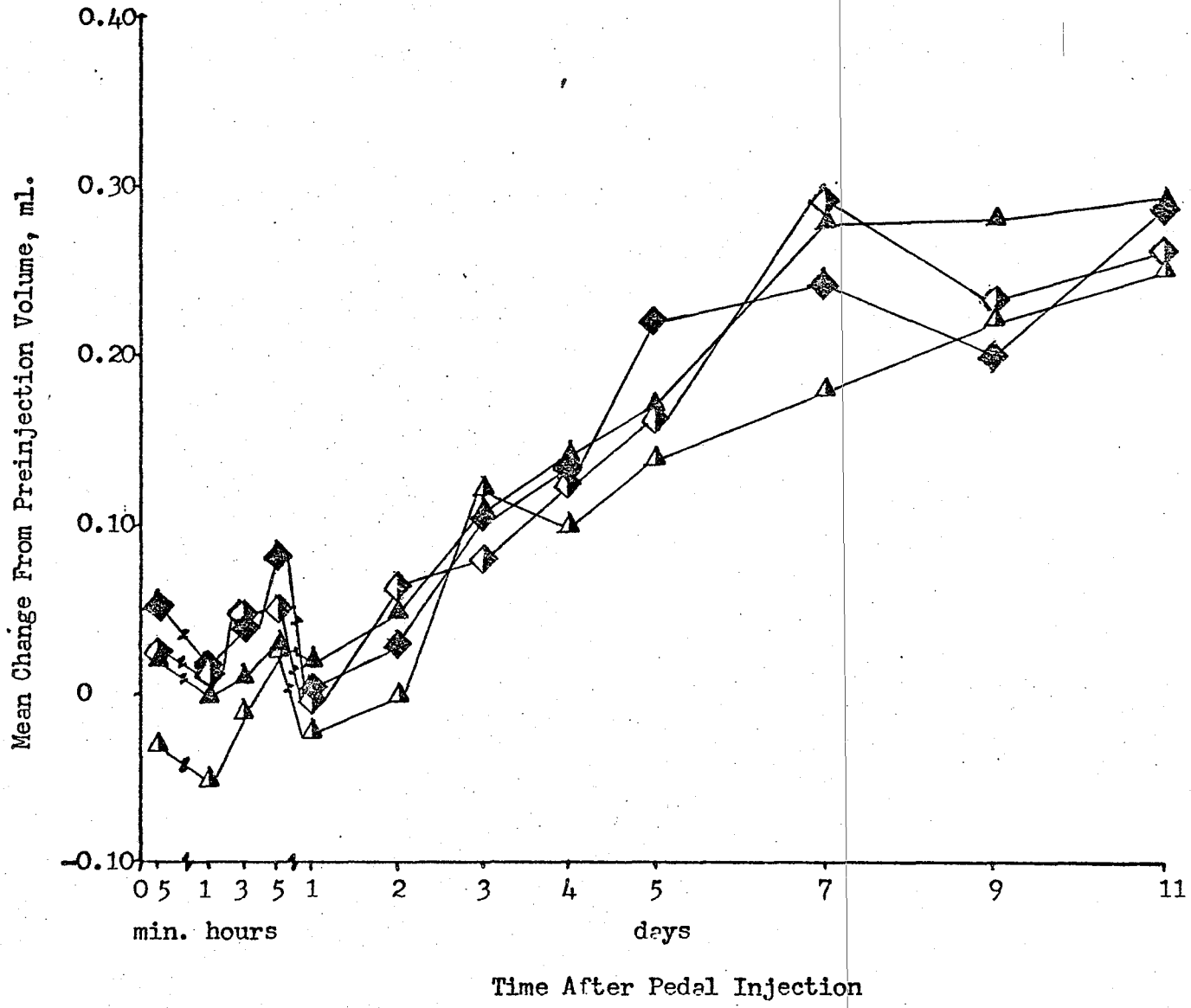
Values indicate change from preinjection volume, ml.

◆ whole shrimp

◇ deproteinated shrimp

▲ whole loligo

△ deproteinated loligo


0.40
0.30
0.20
0.10
0
-0.10
Mean Change From Preinjection Volume, ml.
0 5 1 3 5 1 2 3 4 5 7 9 11
min. hours
days
Time After Pedal Injection

ficance at the above mentioned peak times was $p < 0.001$. The inflammation produced by the two above forms of loligo pen material persisted longer than that produced by carrageenin, e.g. whole loligo pen material inflammation persisted through +4 days, while deproteinated material inflammation persisted through +5 days (see Table IX and Figure 20). Whole shrimp and deproteinated shrimp material produced a peak of swelling of +0.51 ml. at +5 hrs. (about 60% of the swelling produced by carrageenin at the same time), and deproteinated shrimp material produced a peak of swelling of +0.42 ml. at +3 hrs. (about 46% of the swelling produced by carrageenin at the same time). Probability levels of significance for the two materials at the peak times was $p < 0.001$. While the two forms of loligo materials produced an inflammation which persisted longer than the inflammation produced by carrageenin, whole shrimp material produced a type of inflammation which was detectable through +9 days. Deproteinated shrimp material produced an inflammation which lasted through +2 days (see Table IX, Figure 21). Whole cotton produced only a sligh degree of inflammation which was significantly different from the saline vehicle control at +5 hrs. and at +1 day (see Table IX). When the whole forms in this study are compared to one another for their phlogistic capacity, it appears that whole shrimp had more phlogistic capacity than does whole loligo pen material (see Table IX and Figure 23). When the deproteinated forms in this study are compared to one another for their phlogistic capacity, it appears that deproteinated shrimp had more phlogistic ability than does deproteinated loligo pen material (see Table IX and Figure 24). There was no evidence of swelling or

of secondary lesions in the contralateral paws of the animals used in this study (see Table X and Figures 25-27).

Short-term, single-dose drug studies:

The results summarized in Tables XI-XIV and in Figures 28-31 are from a one-level drug study which was performed in order to determine possible mechanisms of action whereby chitinous materials induce inflammation. Whole velella float material was chosen as the type of chitinous material to be studied because of its ready availability. Theoretically, the information gathered concerning whole velella-induced inflammation should be representative of most whole-forms of chitinous materials.

Velella-induced pedal edema was reduced by premedication with phenylbutazone at +3 hrs. and at +5 hrs. by 31% and 33%, respectively (see Table XI and Figure 28). Indomethacin and chlorpromazine were also effective in reducing velella-induced inflammation at +3 hrs. and at +5 hrs. by 26% and 33%, reapectively. Chlorpromazine reduced velella-induced inflammation at +3 hrs. and +5 hrs. by 36% and 40%, respectively (see Table XI and Figure 28). Since the previous work has shown that there is no statistically significant degree of swelling in the contralateral paw, the effectiveness of these drugs upon contralateral paw volume was expected not to be significant (see Table XII and Figure 29). Table XIII and XIV and Figure 30 and 31 show carrageenin data run concurrently. Phenylbutazone was effective in reducing carrageenin-induced pedal edema at +3 hrs. by 16% (see Table XIII and Figure 30). Indomethacin and chlorpromazine were somewhat more effective in retarding carrageenin-induced inflammation at +1 hr. by 32% and at +3 hrs. by 30%. Chlorproma-

Table XI: Effects of phenylbutazone, indomethacin, and chlorpromazine on mean injected paw volumes of rats with velella-induced inflammation (phlogiston vehicle: 0.9% saline)

Drug treatment at -1 hr.	Oral dosage, mg./kg.	N	Preinj. volume, ml.	Change from preinjection volume, ml. (± 1 SEM) +5 min.	+1 hr.	+3 hr.	+5 hr.
Agar vehicle	----	20	1.17	+0.17 (0.03)	+0.18 (0.03)	+0.51 (0.05)	+0.45 (0.03)
Phenylbutazone	100	20	1.15	+0.15 (0.03)	+0.18 (0.04)	+0.35[a] (0.05)	+0.30[b] (0.03)
Indomethacin	10	20	1.13	+0.16 (0.03)	+0.16 (0.03)	+0.37[a] (0.05)	+0.30[b] (0.03)
Chlorpromazine	100	20	1.19	+0.12 (0.02)	+0.18 (0.03)	+0.32[b] (0.04)	+0.27[c] (0.03)

Significantly different from the corresponding "agar vehicle" value:
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. For all other values, $p > 0.05$.

Table XII: Effects of phenylbutazone, indomethacin, and chlorpromazine on mean contralateral paw volumes of rats with velella-induced inflammation (phlogiston vehicle: 0.9% saline)

Drug treatment at -1 hr.	Oral dosage, mg./kg.	N	Preinj. volume, ml.	Change from preinjection volume, ml. (± 1 SEM)			
				+5 min.	+1 hr.	+3 hr.	+5 hr.
Agar vehicle	---	20	1.14	+0.00 (0.02)	+0.00 (0.01)	-0.01 (0.01)	-0.04 (0.01)
Phenylbutazone	100	20	1.10	-0.01 (0.02)	-0.01 (0.02)	+0.00 (0.02)	-0.03 (0.02)
Indomethacin	10	20	1.11	-0.01 (0.02)	+0.02 (0.02)	-0.01 (0.02)	-0.02 (0.02)
Chlorpromazine	100	20	1.16	+0.03 (0.02)	+0.04 (0.02)	+0.03 (0.02)	+0.01[a] (0.02)

Significantly different from the corresponding "agar vehicle" value:
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. For all other values, $p > 0.05$.

Table XIII: Effects of phenylbutazone, indomethacin, and chlorpromazine on mean injected paw volumes of rats with carrageenin-induced inflammation (phlogiston vehicle: 0.9% saline)

Drug treatment at -1 hr.	Oral dosage, mg./kg.	N	Preinj. volume, ml.	Change from preinjection volume, ml. (± 1SEM)			
				+5 min.	+1 hr.	+3 hr.	+5 hr.
Agar vehicle	----	20	1.15	+0.17 (0.02)	+0.62 (0.04)	+0.92 (0.05)	+0.82 (0.04)
Phenylbutazone	100	20	1.21	+0.18 (0.02)	+0.52 (0.04)	+0.76[a] (0.05)	+0.70 (0.03)
Indomethacin	10	20	1.17	+0.09[b] (0.02)	+0.38[c] (0.03)	+0.72[b] (0.04)	+0.71 (0.05)
Chlorpromazine	100	20	1.14	+0.17 (0.02)	+0.53 (0.04)	+0.73[b] (0.05)	+0.65[b] (0.04)

Significantly different from the corresponding "agar vehicle" value:
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. For all other values $p > 0.05$.

Table XIV: Effects of phenylbutazone, indomethacin, and chlorpromazine on mean contralateral paw volumes of rats with carrageenin-induced inflammation (phlogiston vehicle: 0.9% saline)

Drug treatment at -1 hr.	Oral dosage, mg./kg.	N	Preinj. volume, ml.	Change from preinjection volume, ml. (± 1SEM)			
				+5 min.	+1 hr.	+3 hr.	+5 hr.
Agar vehicle	----	20	1.12	+0.00 (0.01)	+0.00 (0.02)	-0.02 (0.02)	-0.03 (0.02)
Phenylbutazone	100	20	1.16	+0.00 (0.02)	+0.00 (0.02)	+0.02 (0.02)	-0.01 (0.01)
Indomethacin	10	20	1.14	-0.03 (0.02)	-0.04 (0.01)	-0.06 (0.02)	-0.05 (0.02)
Chlorpromazine	100	20	1.10	-0.02 (0.02)	+0.03 (0.02)	-0.01 (0.01)	+0.00 (0.02)

Significantly different from the corresponding "agar vehicle" value: $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$. For all other values, $p > 0.05$.

Fig. 28 - Effects of phenylbutazone, indomethacin, and chlorpromazine on mean injected paw volumes of rats with velella-induced inflammation.

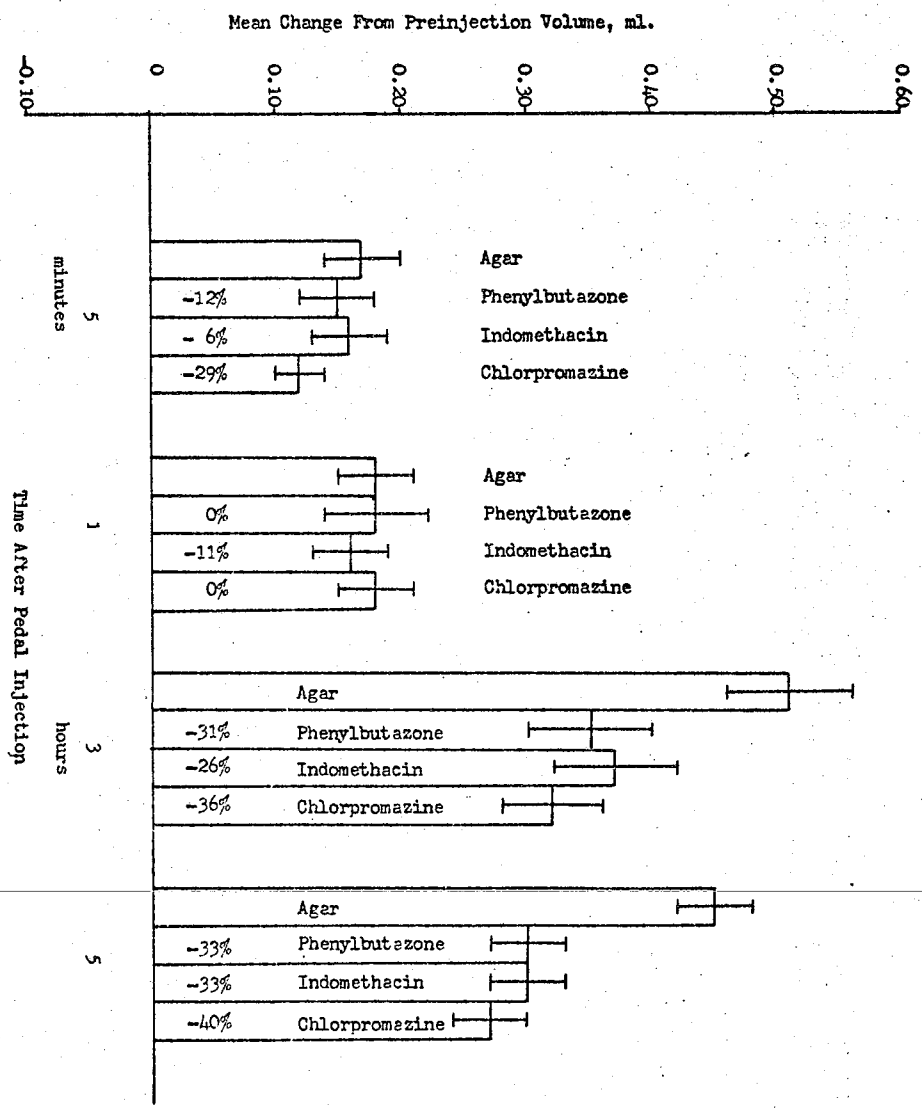

Mean Change From Preinjection Volume, ml.
-0.10
0
0.10
0.20
0.30
0.40
0.50
0.60
Time After Pedal Injection
5 minutes
Agar
-12% Phenylbutazone
- 6% Indomethacin
-29% Chlorpromazine
1 hour
Agar
0% Phenylbutazone
-11% Indomethacin
0% Chlorpromazine
3 hours
Agar
-31% Phenylbutazone
-26% Indomethacin
-36% Chlorpromazine
5 hours
Agar
-33% Phenylbutazone
-33% Indomethacin
-40% Chlorpromazine

Fig. 29 - Effects of phenylbutazone, indomethacin, and chlorpromazine on mean contralateral paw volumes of rats with velella-induced inflammation.

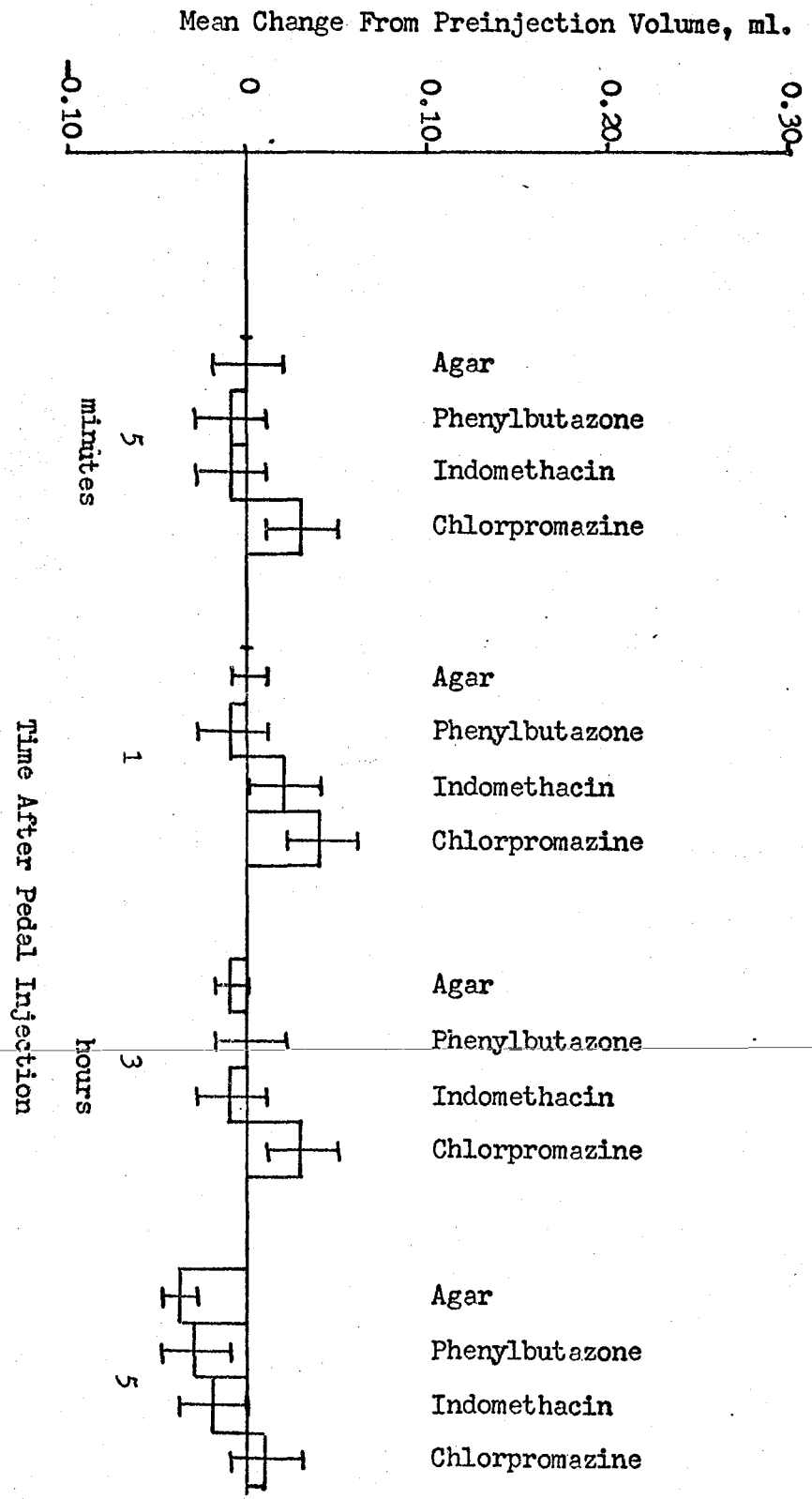

Mean Change From Preinjection Volume, ml.
-0.10
0
0.10
0.20
0.30
Time After Pedal Injection
5 minutes
1 hours
3 hours
5 hours
Agar
Phenylbutazone
Indomethacin
Chlorpromazine
Agar
Phenylbutazone
Indomethacin
Chlorpromazine
Agar
Phenylbutazone
Indomethacin
Chlorpromazine
Agar
Phenylbutazone
Indomethacin
Chlorpromazine

Fig. 30 - Effects of phenylbutazone, indomethacin, and chlorpromazine on mean injected paw volumes of rats with carrageenin-induced inflammation.

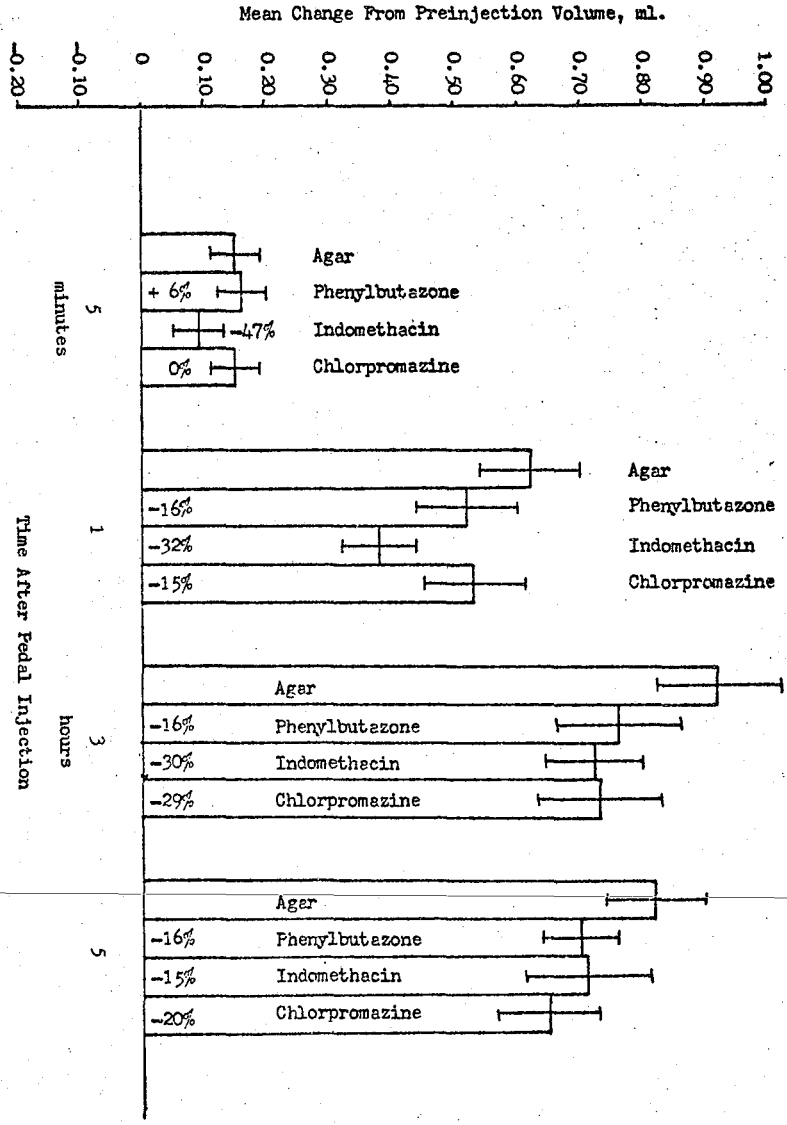
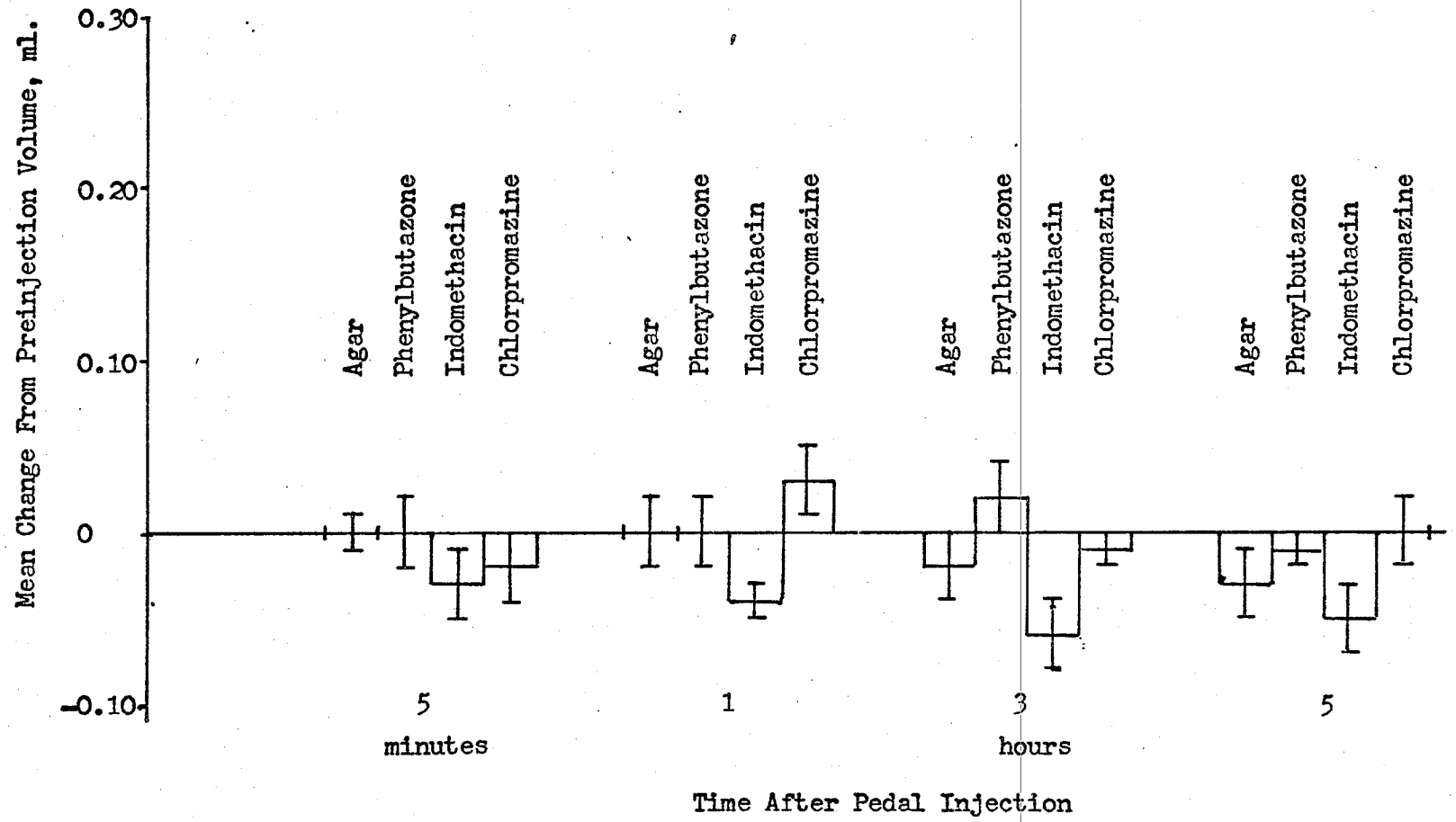

Mean Change From Preinjection Volume, ml.
-0.20
-0.10
0
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
Time After Pedal Injection
5 minutes
Agar
+ 6%
Phenylbutazone
-47%
Indomethacin
0%
Chlorpromazine
1 hours
Agar
-16%
Phenylbutazone
-32%
Indomethacin
-15%
Chlorpromazine
3 hours
Agar
-16%
Phenylbutazone
-30%
Indomethacin
-29%
Chlorpromazine
5 hours
Agar
-16%
Phenylbutazone
-15%
Indomethacin
-20%
Chlorpromazine

Fig. 31 - Effects of phenylbutazone, indomethacin, and chlorpromazine on mean contralateral paw volumes of rats with carrageenin-induced inflammation.


Mean Change From Preinjection Volume, ml.
0.30
0.20
0.10
0
-0.10
Agar
Phenylbutazone
Indomethacin
Chlorpromazine
Agar
Phenylbutazone
Indomethacin
Chlorpromazine
Agar
Phenylbutazone
Indomethacin
Chlorpromazine
Agar
Phenylbutazone
Indomethacin
Chlorpromazine
5
minutes
1
3
hours
5
Time After Pedal Injection

zine reduce carrageenin-induced inflammation at +3 hrs. by 29% and at +5 hrs. by 20% (see Table XIII and Figure 30). As with whole velella-induced inflammation, there was no statistically significant drug effect in the contralateral paws (see Table XIV and Figure 31).

The results summarized in Tables XV-XVIII and in Figures 32-35, are from another study which was performed in order to further delineate possible mechanisms of action whereby chitinous materials induce inflammation. Whole velella float material was again chosen to be examined as an example of chitinous material. The only difference between this study and the previous one was the type of drugs studied: aspirin, hydrocortisone, and cryogenine.

Velella-induced inflammation was significantly reduced with aspirin at +1 hr. by 50%, at +3 hrs. by 49%, and at +5 hrs. by 23%. Velella induced inflammation was also significantly reduced with hydrocortisone at +3 hrs. by 34%. Velella-induced inflammation was not significantly reduced with cryogenine (see Table XV and Figure 32). There was no statistically significant amount of swelling in the contralateral paws, so no measure of drug effectiveness could be obtained or expected (see Table XVI and Figure 33). Table XVII and XVIII and Figure 34 and 35 show carrageenin data run concurrently. Carrageenin-induced inflammation was significantly reduced with aspirin at +1 hr. by 44%, and at +3 hrs. by 41%, and at +5 hrs. by 27%. Carrageenin-induced inflammation was also significantly reduced with hydrocortisone at +3 hrs. by 30%, and with cryogenine at +3 hrs. by 28% (see Table XVII and Figure 34). As with the whole velella material, there was no statistically significant amount of swelling induced by carrageenin in the contralateral paw (see Table XIII and Figure 35).

Table XV: Effects of aspirin, hydrocortisone, and cryogenine on mean injected paw volumes of rats with velella-induced inflammation (phlogiston vehicle: 0.9% saline)

Drug treatment at -1 hr.	Oral dosage, mg./kg.	N	Preinj. volume, ml.	Change from preinjection volume, ml. (± 1 SEM) +5 min.	+1 hr.	+3 hr.	+5 hr.
Agar vehicle	----	10	1.12	+0.22 (0.02)	+0.26 (0.04)	+0.57 (0.06)	+0.48 (0.04)
Aspirin	300	10	1.12	+0.13[b] (0.02)	+0.13[b] (0.03)	+0.29[b] (0.04)	+0.35 (0.08)
Hydrocortisone	20	10	.98	+0.18 (0.06)	+0.28 (0.06)	+0.38[a] (0.04)	+0.41 (0.04)
Cryogenine	100	10	1.15	+0.20 (0.04)	+0.22 (0.05)	+0.47 (0.06)	+0.52 (0.06)

Significantly different from the corresponding "agar vehicle" value:
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. For all other values, $p > 0.05$.

Table XVI: Effects of aspirin, hydrocortisone, and cryogenine on mean contralateral paw volumes of rats with velella-induced inflammation (phlogiston vehicle: 0.9% saline)

Drug treatment at -1 hr.	Oral dosage mg./kg.	N	Preinj. volume, ml.	Change from preinjection volume, ml. (± 1 SEM)			
				+5 min.	+1 hr.	+3 hr.	+5 hr.
Agar vehicle	----	10	1.06	+0.05 (0.03)	+0.01 (0.02)	+0.00 (0.02)	-0.04 (0.02)
Aspirin	300	10	1.07	-0.05[b] (0.02)	-0.05 (0.03)	-0.05 (0.02)	-0.05 (0.02)
Hydrocortisone	20	10	1.02	+0.01 (0.05)	+0.02 (0.03)	-0.03 (0.03)	+0.07 (0.11)
Cryogenine	100	10	1.06	-0.01 (0.02)	-0.05 (0.03)	-0.04 (0.02)	-0.09 (0.03)

Significantly different from the corresponding "agar vehicle" value:
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. For all other values $p > 0.05$.

Table XVII: Effects of aspirin, hydrocortisone, and cryogenine on mean injected paw volumes of rats with carrageenin-induced inflammation (phlogiston vehicle: 0.9% saline)

Drug treatment at -1 hr.	Oral dosage, mg./kg.	N	Preinj. volume, ml.	Change from preinjection volume, ml. (± 1 SEM) +5 min.	+1 hr.	+3 hr.	+5 hr.
Agar vehicle	----	10	1.13	+0.16 (0.03)	+0.57 (0.08)	+1.00 (0.07)	+0.88 (0.07)
Aspirin	300	10	1.10	+0.10 (0.03)	+0.32[a] (0.06)	+0.59[b] (0.08)	+0.65[a] (0.05)
Hydrocortisone	20	10	1.13	+0.17 (0.02)	+0.48 (0.05)	+0.70[b] (0.07)	+0.70 (0.06)
Cryogenine	100	10	1.10	+0.16 (0.03)	+0.52 (0.05)	+0.72[a] (0.11)	+0.70 (0.07)

Significantly different from the corresponding "agar vehicle" value: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. For all other values, $p > 0.05$.

Table XVIII: Effects of aspirin, hydrocortisone, and cryogenine on mean contralateral paw volumes of rats with carrageenin-induced inflammation (phlogiston: 0.9% saline)

Drug treatment at -1 hr.	Oral dosage, mg./kg.	N	Preinj. volume, ml.	Change from preinjection volume, ml. (± 1 SEM) +5 min.	+1 hr.	+3 hr.	+5 hr.
Agar vehicle	----	10	1.06	+0.04 (0.03)	+0.01 (0.03)	-0.01 (0.02)	-0.02 (0.03)
Aspirin	300	10	1.05	+0.07[b] (0.02)	-0.07 (0.03)	-0.06 (0.01)	-0.06 (0.03)
Hydrocortisone	20	10	1.04	+0.01 (0.03)	+0.02 (0.04)	-0.01 (0.02)	-0.02 (0.04)
Cryogenine	100	10	1.02	+0.00 (0.02)	-0.05 (0.01)	-0.05 (0.01)	-0.06 (0.02)

Significantly different from the corresponding "agar vehicle" value:
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. For all other values, $p > 0.05$.

Fig. 32 - Effects of aspirin, hydrocortisone, and cryogenine on mean injected paw volumes of rats with velella-induced inflammation.

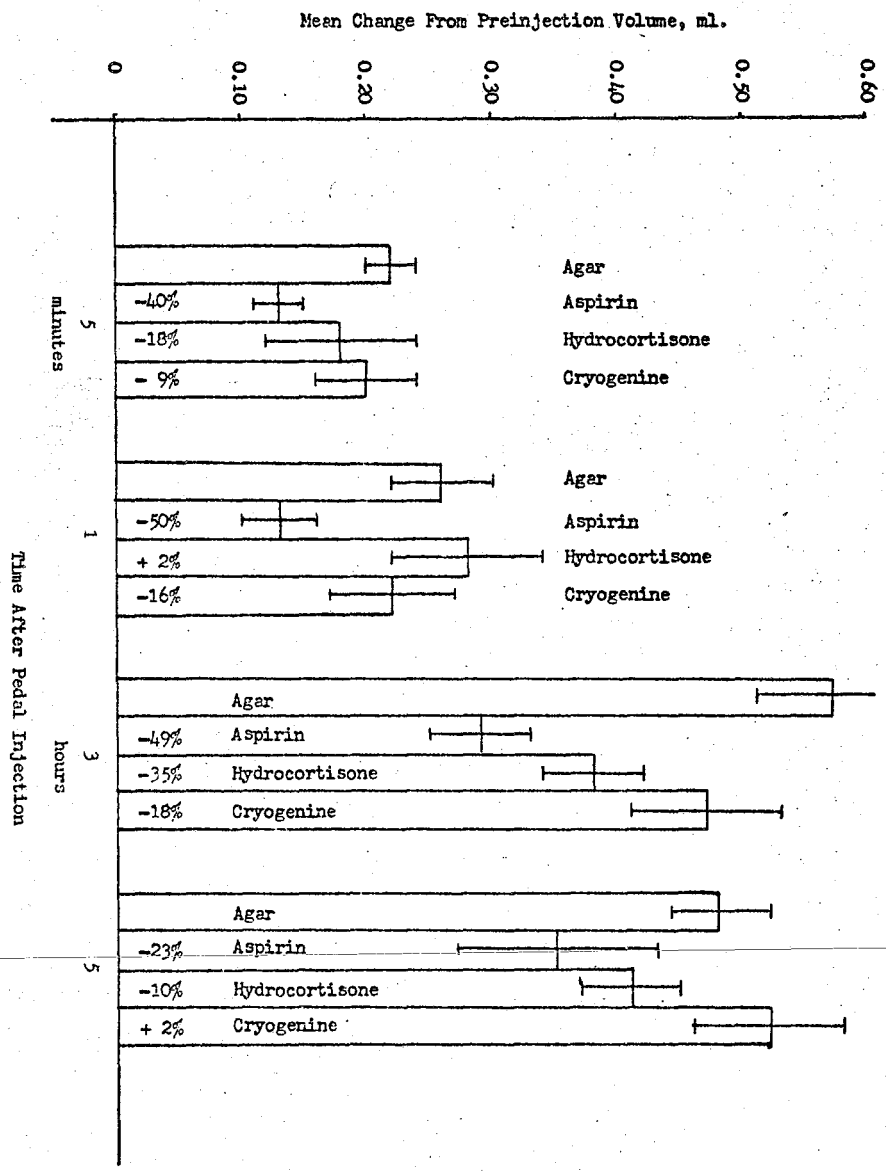

Mean Change From Preinjection Volume, ml.
0
0.10
0.20
0.30
0.40
0.50
0.60
Time After Pedal Injection
5 minutes
Agar
-40%
Aspirin
-18%
Hydrocortisone
- 9%
Cryogenine
1 hour
Agar
-50%
Aspirin
+ 2%
Hydrocortisone
-16%
Cryogenine
3 hours
Agar
-49%
Aspirin
-35%
Hydrocortisone
-18%
Cryogenine
5 hours
Agar
-23%
Aspirin
-10%
Hydrocortisone
+ 2%
Cryogenine

Fig. 33 - Effects of aspirin, hydrocortisone, and cryogenine on mean contralateral paw volumes of rats with velella-induced inflammation.

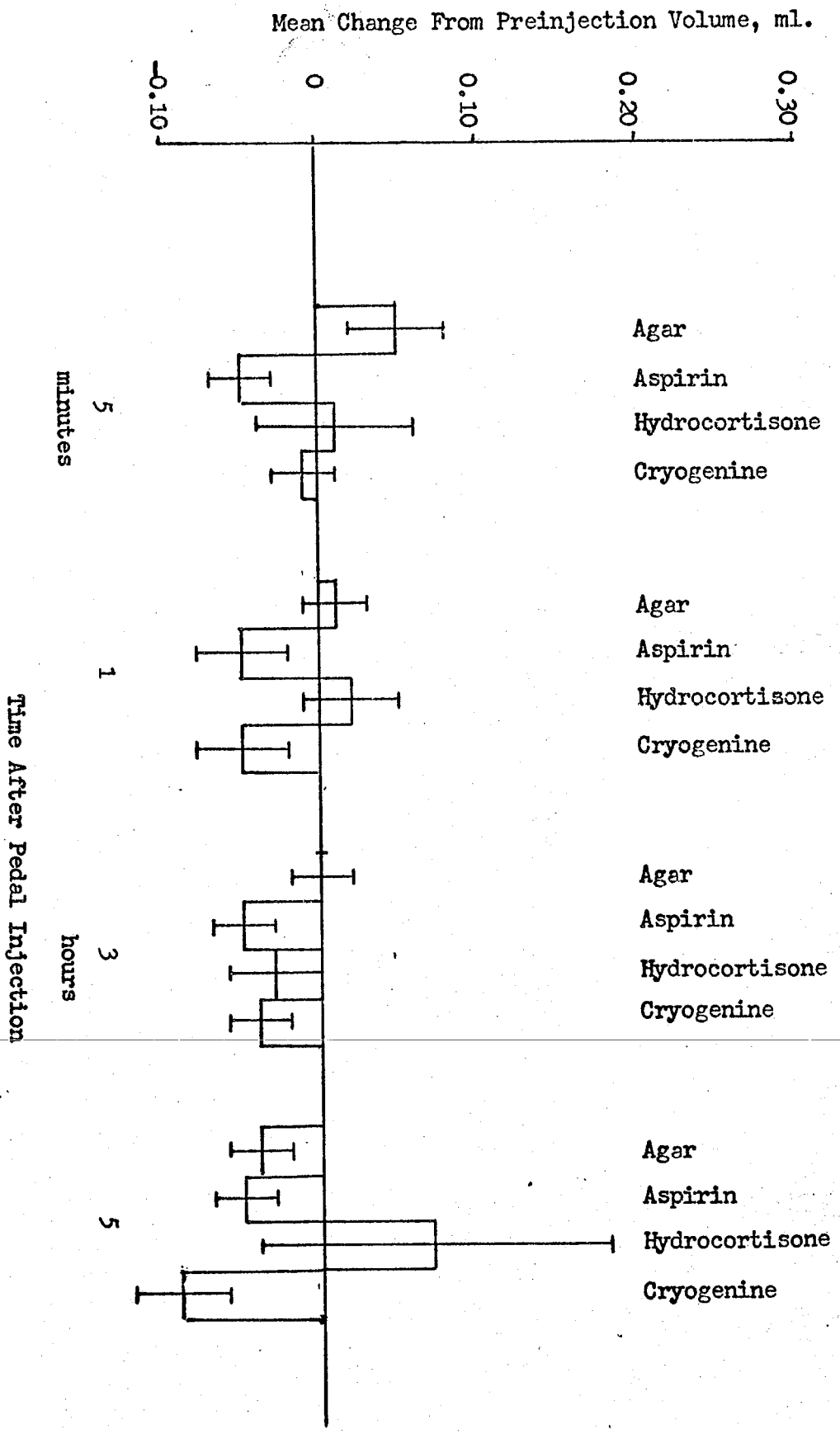

Mean Change From Preinjection Volume, ml.
-0.10
0
0.10
0.20
0.30
Time After Pedal Injection
5 minutes
1 hour
3 hours
5
Agar
Aspirin
Hydrocortisone
Cryogenine
Agar
Aspirin
Hydrocortisone
Cryogenine
Agar
Aspirin
Hydrocortisone
Cryogenine
Agar
Aspirin
Hydrocortisone
Cryogenine

Fig. 34 - Effects of aspirin, hydrocortisone, and cryogenine on mean injected paw volumes of rats with carrageenin-induced inflammation.

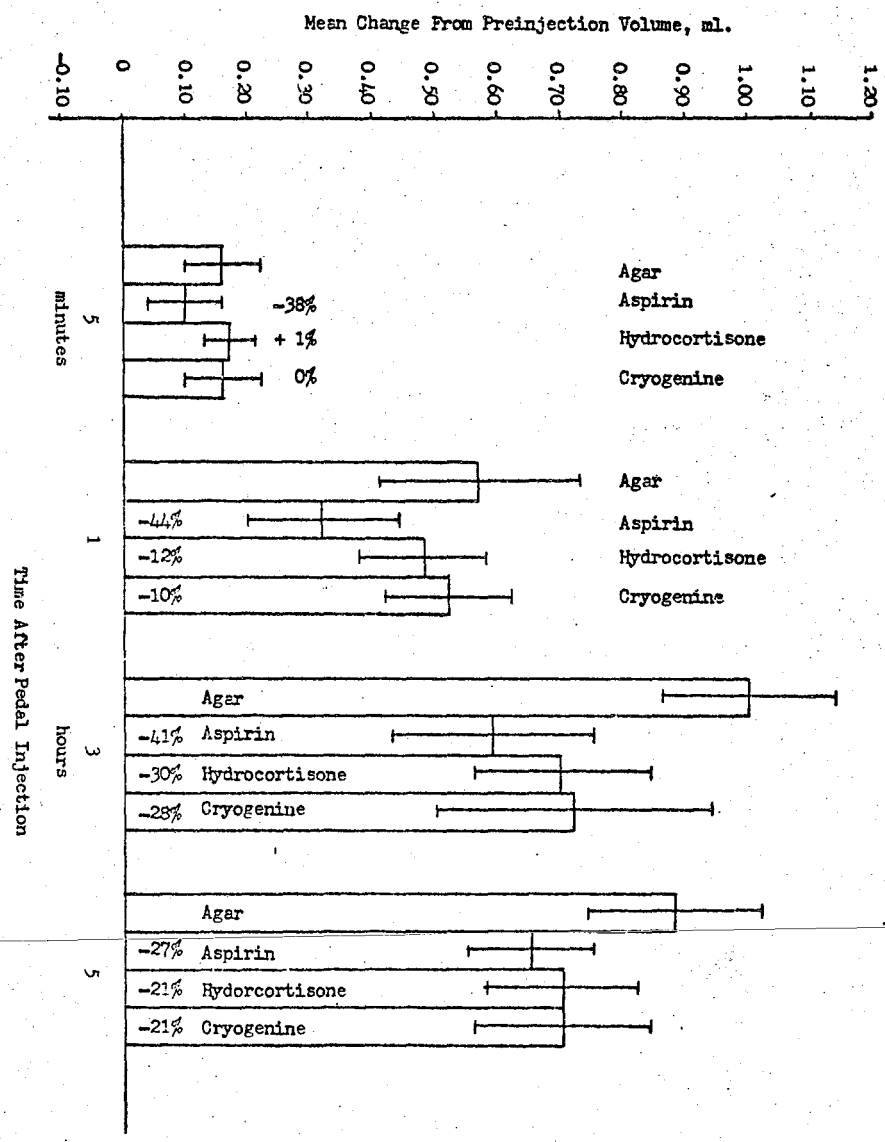
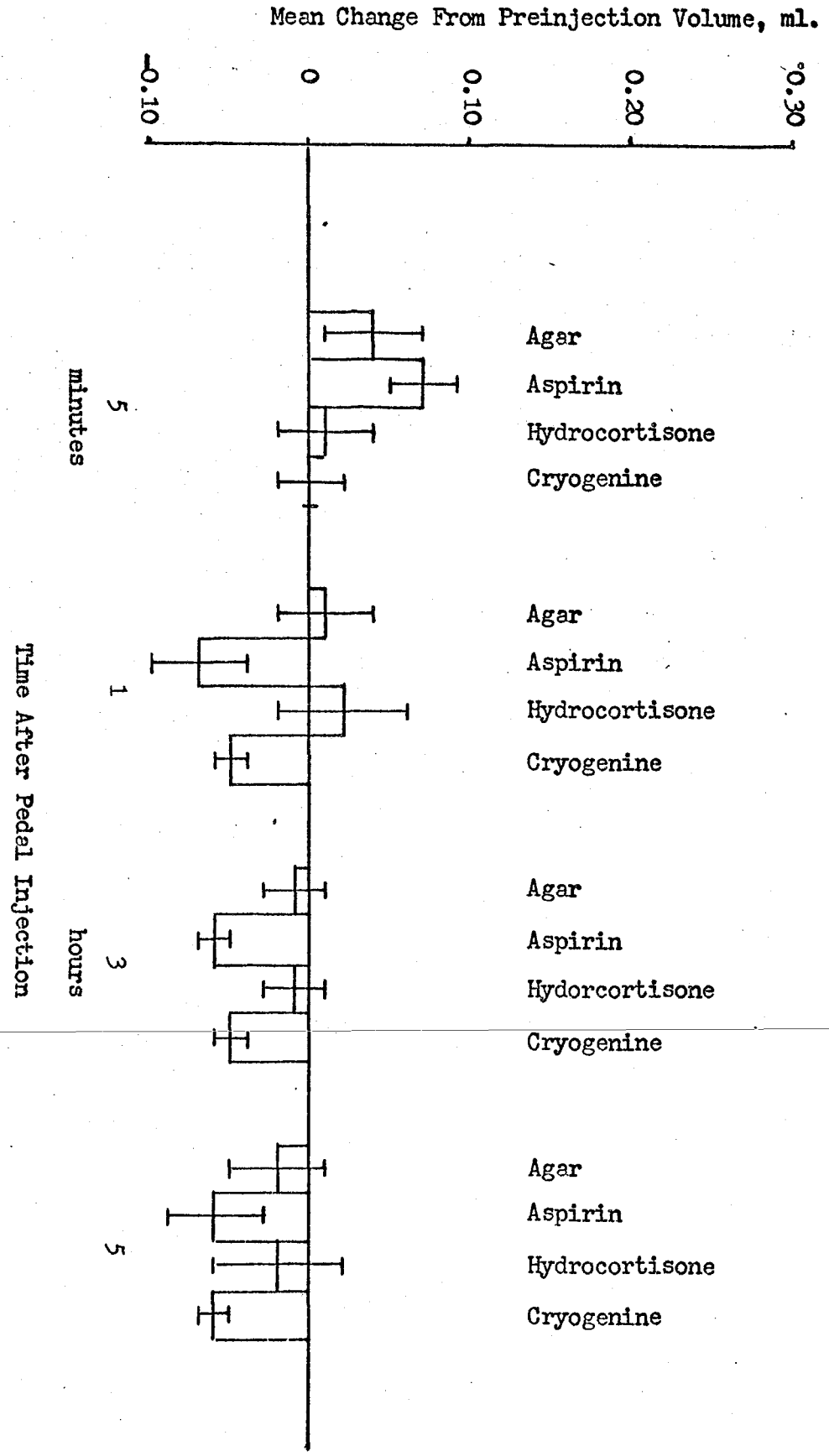

Mean Change From Preinjection Volume, ml.
-0.10
0
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
1.10
1.20
Time After Pedal Injection
5 minutes
1 hours
3 hours
5 hours
Agar
Aspirin
Hydrocortisone
Cryogenine
-38%
+ 1%
0%
-44%
-12%
-10%
-41%
-30%
-28%
-27%
-21%
-21%

Fig. 35 - Effects of aspirin, hydrocortisone, and cryogenine on mean contralateral paw volumes of rats with carrageenin-induced inflammation.


Mean Change From Preinjection Volume, ml.
-0.10
0
0.10
0.20
0.30
Time After Pedal Injection
5 minutes
1 hours
3 hours
5 hours
Agar
Aspirin
Hydrocortisone
Cryogenine
Agar
Aspirin
Hydrocortisone
Cryogenine
Agar
Aspirin
Hydorcortisone
Cryogenine
Agar
Aspirin
Hydrocortisone
Cryogenine

DISCUSSION

The lack of suitable experimental models in animals for inflammatory diseases has hindered the pharmacological search for truly effective drugs for the treatment of these diseases in man. Pharmacologists continue to use inadequate models to select usually inadequate drugs. With this in mind, the present research was directed towards determining:

(i) whether or not invertebrate chitinous material would be capable of inducing an inflammatory response and if so,

(ii) what the possible mechanism(s) of action in this induction process might be.

To accomplish the objectives outlined above, several series of experiments were conducted. The first series of experiments were performed in order to determine whether or not chitinous materials were indeed capable of inducing an inflammatory response, and if so, whether this response was similar to that induced by carrageenin (acute response) or by mycobacterium adjuvant (acute and sustained response). Although the only chitinous forms studied in these initial series of experiments were those which were derived from Velella lata, the information collected can be extrapolated to all invertebrate chitinous materials since it is only the protein

of the N-acetylglucosamine-protein complex which differs from one species of invertebrate to another). Velella lata was chosen to serve as the prototype chitin material in this initial series of experiments because it was supplied to us first and in greater abundance than any of the other invertebrate chitins. The results of the experiments which were aimed toward determining the degree of similarity between chitin-induced inflammation and mycobacterium adjuvant-induced inflammation are depicted in Tables I and II and in Figures 1 - 6. The results of the experiments aimed toward depicting the degree of similarity between chitin-induced inflammation and carrageenin-induced inflammation are depicted in Tables III and IV and in Figures 7 - 13.

The polyarthritic condition induced in the rat by the intradermal injection of mycobacterium adjuvant into either the hind paw or tail is probably the most widely used "model" for rheumatoid disease in screening programs for anti-inflammatory drugs (17). This arthritic condition has been suggested variously to be due to: (i) a delayed hypersensitivity response to mycobacterial antigen (31a) and/or (ii) an autoimmune disease whereby the responsible agent alters collagen (32). Some symptoms of the polyarthritis induced by mycobacterium adjuvant in the rat are as follows: swelling and erythema of the injected as well as the contralateral paw, loss of the use of the ankle joints in these paws, development of secondary lesions in the tail, ears, and nose, and an early and abrupt decrease in body weight (33). The time course is biphasic in nature (34). The first phase is represented by an immediate swelling of the

injected hind paw which peaks in four days and clearly recedes between the seventh and eighth days. Clinical features of this phase include erythema, edema, ulceration, and a purulent discharge at the site of the inoculation. The second pahse is delayed, beginning around the tenth post-inoculation day and continuing through the thirtieth day. This second phase can be roughly divided into early (11th - 16th day) and late (18th - 30th day) stages based on histological changes. While the first phase involves the inoculated area only, the second phase involves all four paws (swelling of the contralateral and fore-paws is usually seen around the twentieth day post-innoculation) and includes various mucocutaneous lesions of the tail, eyes, ears, genitals, and skin. There is also an intense connective tissue reaction (fibro-/and osteoplasia) which produces a severe and disabling ankylotic condition. There is a general decrease in whole body weight during the course of the second phase. This description indicates that while the second phase includes an acute inflammatory component, the whole overall picture suggests a chronic inflammatory state.

When whole, deproteinated, and deacetylated forms of Velella lata were suspended in mineral oil and injected in the same manner as was the mycobacterium adjuvant, some swelling was noted in the injected paws which was statistically significant from the vehicle control, but the time sequence for the swelling did not appear to follow any consistent pattern. Futhermore, the swelling was only about one-sixth of that produced by the mycobacterium. In addition, there was no evidence of any systemic disease in animals injected

with velella materials as compared to those animals receiving the mycobacterium adjuvant (e.g. there was no contralateral paw swelling or the development of mucocutaneous lesions). Therefore, one can conclude that invertebrate chitinous materials lack adjuvant-like phlogistic potential.

Carrageenin is a mixture of polysaccharides composed of sulfated galactose units and derived from Irish sea moss (Chrondrus crispus). Its use as an edemogen was introduced by Winter et al., (35) who documented the acute localized inflammatory edema produced when the substance was injected into the hind paws of rats. The actual development of pedal edema has been described as a biphasic event (36). The initial phase of the edema occurs about one hour after injection and has been attributed to the release of histamine, serotonin, and kinin-like substances. The second phase of edema, which occurs about three hours postinjection and which is about three times as great as the initial phase, has been attributed to prostaglandin-like substances (37). The recognition of different mediators for different phases of the edema has important implications for interpreting the mechanism of action of drugs. It should be pointed out at this time, however, that the identity of these mediators which are claimed to be responsible for the evolution of the edema induced by carrageenin is by no means firmly established (37).

When the velella materials were suspended in 0.9% saline and injected in the same manner as was the carrageenin, a rapid-onset carrageenin-like inflammation was produced, but this inflammation was not as intense as that seen with carrageenin. Futhermore, this

inflammation persisted (unlike carrageenin) through the twentieth day of the observation period (see Table II and Figures 7 - 9). Like carrageenin, there was no evidence of the swelling or of the development of secondary lesions in the contralateral paws (see Table IV and Figures 11 - 13).

From these studies, it became apparent that invertebrate chitinous materials do possess phlogistic potential (i.e., can cause an inflammatory response) and that the response produced is more similar to carrageenin-induced inflammation than to mycobacterium adjuvant-induced inflammation. These initial studies confirm one of the hypothesis set forth in the introduction -- invertebrate chitinous materials do possess phlogistic ability.

Having documented the phlogistic potential of invertebrate chitins, the next step in this study was to determine the minimum molecule(s) necessary for inducing this inflammation, i.e., whether or not it was the complete glycoprotein complex, the carbohydrate moeity alone, or of the protein alone which was responsible for the induction of the inflammatory response. Each species of invertebrate chitin studied will now be discussed individually. By studying the results listed in Tables V - X, and illustrated in Figures 14 - 27, a summary table was constructed (Table XIX). From this table, the following statements concerning the phlogistic capacities of the various forms of invertebrate chitinous materials can be made: (i) whole velella material has more phlogistic capacity than partly de-acetylated velella material, and partly de-acetylated velella material has more phlogistic capacity than deproteinated

Fig. 35 - Effects of aspirin, hydrocortisone, and cryogenine on mean contralateral paw volumes of rats with carrageenin-induced inflammation.

Table XIX: Phlogistic capacities of the different forms of invertebrate chitins

	Percent of carrageenin-induced inflammation		
	Velella	Shrimp	Loligo
Whole forms (glycoprotein complexes: poly N-acetylglucosamine + protein)	51	56	49
Partly deproteinated forms (poly N-acetylglucosamine)	11	49	22
Partly deacetylated forms (glucosamine)	35	36	46

velella material: (ii) whole loligo material has more phlogistic capacity than partly de-acetylated loligo material, and partly de-acetylated loligo has more phlogistic capacity than deproteinated loligo material; and (iii) whole shrimp material has more phlogistic capacity than the deproteinated shrimp material, and deproteinated shrimp material has more phlogistic capacity than the deacetylated shrimp material.

A trend can be seen for the velella and loligo materials. In both cases, the whole forms dominate in phlogistic ability, followed by the partly deacetylated and then the deproteinated forms. This may mean: (i) that it is the glycoprotein complex that is most effective in inducing inflammation, (ii) that it is probably the protein moeity which is most responsible for the inflammatory effect, and (iii) that masking of the glucosamine by the acetyl group may actually retard the inflammatory effect of the glucosamine alone. The only difference between the deproteinated and partly deacetylated forms is the lack of the acetyl groups on the glucosamine in the partly deacetylated forms; both forms already have had the protein removed.

It was hoped that the same trend would be observed with the shrimp material, i.e., that the whole material would predominate in phlogistic ability followed by the deacetylated form and then by the deproteinated forms, but such was not the case. Whole shrimp material was dominant in phlogistic ability, but unexpectedly the deproteinated shrimp possessed more phlogistic ability than the deacetylated shrimp material. Therefore, this could mean that while the shrimp glycoprotein complex is most phlogistic, the carbohydrate moeity is at least partially

responsible for the inflammatory effect since in this case much phlogistic capacity is retained even when the shrimp is in the deproteinated form. Removing the acetyl group from the glucosamine did not improve the phlogistic ability of the shrimp.

Since the information gathered from studying the velella, loligo, and shrimp chitins did not yield consisten results, only a few general statements concerning invertebrate chitin inflammation and its mechanism of action can be made: (i) invertebrate chitins do indeed produce inflammation; (ii) of the three forms studied, velella possesses the greatest phlogistic ability, then shrimp, and finally loligo; (iii) chitin-induced inflammation is grossly similar to carrageenin-induced inflammation, but less intense and of somewhat longer duration, and (iv) although the minimum molecule or molecules necessary for inducing chitin inflammation cannot as yet be identified, the whole glycoprotein complex is most phlogistic. More work is needed to adequately assess the roles of the carbohydrate and protein moeities. Cotton, a polymer of glucose, was tested in this research and found to possess only very minimal phlogistic capacity (see Table VII - X). Consequently, we suspect at this time that it is the protein moeity of the carbohydrate-protein complex which plays the significant role in inducing inflammation.

In an attempt to identify the mechanism of action by which invertebrate chitinous material could induce inflammation, short-term single-dose, drug studies were run. Whole velella float material was chosen as the type of chitinous material to be examined in these studies because of its ready availability and its phlogistic capacity.

Theoretically, the information gathered concerning whole velella-induced inflammation should be representative of the whole forms of other chitinous materials.

Since it has been generally agreed that carrageenin-induced pedal edema in the rat is the most reliable exudative model extrapolating experimental data to clinical anti-inflammatory effectiveness (38), and since the type of inflammation induced by carrageenin is somewhat similar to that induced by the invertebrate chitins, this animal model was ideal for the drug studies conducted here. Prototype, clinically effective anti-inflammatory drugs, including phenylbutazone, indomethacin, aspirin, hydrocortisone, and cryogenine, were screened in parallel experiments for their anti-inflammatory effectiveness against both whole velella-induced inflammation and against carrageenin-induced inflammation. Chlorpromazine was studied because it lacks true anti-inflammatory activity in the clinic, yet responds positively in carrageenin animal studies. The carrageenin drug studies provided us with (i) reference data with which the results from the velella drug studies could be compared, and (ii) a possible means of determining indirectly the mechanism(s) of action by which whole chitinous materials can induce inflammation.

Before proceeding to discuss the effectiveness or non-effectiveness of each of the aforementioned drugs, it is first necessary to review briefly the time course study for both carrageenin and velella-induced inflammation as observed in our research. Study of the time-course of carrageenin-induced edema shows that there is a very sharp

increase in edema formation at +1 hr. after subplantar injection. Another increase is noted approximately +3 hr. after injection and is maintained up through +5 hr. These results are consistent with those obtained by De Cato (39). The time course study of our velella-induced edema, unlike that of carrageenin, did not show a sharp rise at +1 hr.; however, like carrageenin, velella did show a sharp rise in edema formation +3 hr. after subplantar injection. This edema also was maintained through +5 hrs. The amount of edema formation was not as intense, however, as that seen with carrageenin. It is important to review these time course studies here because it has been demonstrated that many anti-inflammatory drugs exert their major effect during either the first or the second phase of carrageenin-induced inflammation.

Phenylbutazone was shown to significantly reduce carrageenin-induced inflammation by 16% at +3 hr. and by 15% at +5 hr.; and to significantly reduce whole velella-induced inflammation by 31% at +3 hr. and by 33% at +5 hr. (See Tables XIII and XI and Figures 30 and 28). The mechanism by which phenylbutazone exerts its anti-inflammatory activity against carrageenin-induced inflammation is not thoroughly understood, but it is comparable to the adrenocorticosteroids in experimental models (40).

ACTH, cortisol, and the synthetic analogs of cortisol have the capacity to prevent or suppress the development of the local heat, redness, swelling, and tenderness by which inflammation is recognized at the gross level of observation. At the microscopic level, they inhibit not only the early phenomena of the inflammatory

process (edema, fibrin dposition, capillary dilation, migration of phagocytes to the inflammed area, and phagocytic activity) but also the later manifestations (capillary proliferation, fibroblast proliferation, and deposition of collagen) (41). Although there are generally no accepted explanations for the above facts, many of these observations have obvious therapeutic relevance. Perhaps the most important is simply that the corticosteroids inhibit inflammation whether the inciting agent is mechanical, chemical, or immunological. In clinical terms, the primary cause remains, but the secondary manifestations can be suppressed. One can understand why cortisone might be suggested for a patient with rheumatoid arthritis who is severely ill and has fever, joint swelling, and intense pain.

Cortisol (hydrocortisone) is the principal glucocorticoid secreted by the adrenal cortex. In adrenalectomized rats, De Cato demonstrated (42) that phenylbutazone retained some of its anti-inflammatory properties but there was a shifting of the dose-response curve with the lowest "effective" dose becoming ineffective under these conditions. This could indicate that part of the anti-inflammatory action of phenylbutazone may be mediated through the pituitary-adrenal axis, and hence explain why phenylbutazone's side effects as an anti-inflammatory agent are similar to those of the adrenocorticoids.

Indomethacin was shown significantly to reduce carrageenin-induced inflammation by 32% at +1 hr. and by 30% at +3 hr.; and to reduce velella-induced inflammation by 11% at +1 hr. and by 33% at +3 hr. (see Tables XIII and XI, and Figures 30 and 28, respectively).

Indomethacin has been shown to uncouple oxidative phosphorylation in cartilagenous and hepatic mitochondria, and its anti-inflammatory mechanism of action could be related to this effect (43). It has also been demonstrated to inhibit the motility of polymorphonuclear leucocytes and consequently part of the anti-inflammatory activity could be related to this effect (43).

Chlorpromazine was shown to significantly reduce carrageenin-induced inflammation by 29% at +3 hr. and by 20% at +5 hr.; and to significantly reduce velella-induced inflammation by 36% at +3 hr. and by 40% at +5 hr. (see Tables XIII and XI, and Figures 30 and 28, respectively). Although chlorpromazine is shwon to be effective against carrageenin-induced inflammation, it should be noted that it is even more effective in retarding velella-induced inflammation.

Chlorpromazine has been shown to be anti-inflammatory in a variety of experimental models (44, 45) but it lacks effectiveness in the clinical treatment of inflammation. The variety of pharmacological effects produced by chlorpromazine make it extremely difficult to predict a possible mechanism of action for this compound in this instance. Chang (46) suggested that its anti-histaminic, anti-serotonin and anti-bradykinin activities were not responsible for the inhibition of carrageenin-induced pedal edema since cyproheptadine (a more potent inhibitor of these substances) was relatively inactive. Arrigoni-Martelli et al. (47) suggested that its anti-inflammatory properties were correlated with its hypothermic activity, but this seems doubtful since Garattini et al. (48) demonstrated that meprobamate did not inhibit edema formation caused by carrageenin,

yeast, or dextran when compared at equal hypothermic doses to chlorpromazine. Marckel et al. (49) demonstrated that reserpine and chlorpromazine cause a release of ACTH and that this may account for the anti-inflammatory activity of chlorpromazine. However, reserpine does not inhibit carrageenin-induced inflammation; and De Cato (50) has demonstrated that although chlorpromazine may stimulate the pituitary-adrenal axis, its anti-inflammatory properties cannot be wholly attributable to this phenomena.

It should be noted that the dosage of chlorpromazine used in this study to effect an inhibition of the inflammatory response exceeded its ataractic dose in the rat.

Aspirin was shown to significantly reduce carrageenin-induced inflammation by 44% at +1 hr., by 41% at +3 hr., and by 27% at +5 hr.; and to significantly reduce velella-induced inflammation by 50% at +1 hr., by 49% at +3 hr., and by 23% at +5 hr. (see Tables XVII and XV, and Figures 34 and 32, respectively).

A variety of experimentally induced inflammatory syndromes in animals are suppressed by salicylates. Since a cardinal feauture of inflammation is fluid exudation secondary to increased capillary permeability, attention has been devoted to the possibility that injuries of all types might cause the local tissue release or activation of substances (mediators) that increase capillary permeability. Many substances proposed as mediators are histamine, serotonin, bradykinin, and kallikrein (see Introduction for further discussion on mediators). Salicylates, however, do not supppress bradykinin formation or prevent the inflammation induced by it;

they do not inhibit the kallikrein-bradykinin system; nor do they antagonize histamine or serotonin (51). Salicylates do stabilize the lysosomes and are thought to exert part of their anti-inflammatory effect by preventing the release of hydrolases and proteases during inflammation. Perhaps the main mechanism involved is the non-specific effect of the salicylates to somehow reduce the capillary permeability increased by the inflammatory process (51).

Salicylates also can affect the composition, biosynthesis, and metabolism of connective tissue mucopolysaccharides (52). This factor offers a possible explanation for the anti-inflammatory efficacy of the drug in the treatment of chronic inflammatory disorders.

Hydrocortisone was shown to significantly reduce carrageenin-induced inflammation by 30% at +3 hr.; and to significantly reduce velella-induced inflammation by 34% at +3 hr. (See Tables XVII and XV and Figures 34 and 32, respectively).

As stated earlier, hydrocortisone is the principal glucocorticoid secreted by the adrenal cortex. It has the capacity to suppress the development of inflammation by inhibiting edema formation, fibrin deposition, capillary dilation, migration of phagocytes to the inflammed area, and also to inhibit fibroblast proliferation and the deposition of collagen. Also, as stated earlier, there is no generally accepted explanation of these facts (41). It has been speculated, however, that cortisol suppresses inflammation by promoting the containment not only of cathepsins especially, but also other enzymes within the lysosomal sac (53).

Although it appears that hydrocortisone might be ideal for

treating inflammation resulting from lack of homeostatic control, in reality it is not. The reason for this is that there are serious risks associated with its chronic use: (i) cortisone therapy, once started, may have to be continued for an undetermined time, due to the complications it produces with withdrawl; (ii) with prolonged treatment, cortisone may also affect the body's immune system due to its thymolytic properties; (iii) cortisone injections for the treatment of rheumatoid and osteo-arthritis may cause painless destruction of the afflicted joint (54); (iv) sodium retention on chronic therapy can precipitate hypertension; (v) atrophy of the adrenal cortex can result due to suppression of ACTH; and (vi) wounds heal poorly and patients cannot undergo surgical procedures.

Cryogenine, an alkaloid isolated from Heimia salicifolia, Link and Otto, was shown to significantly reduce carrageenin-induced inflammation by 28% at +3 hr.; it was not shown to significantly reduce velella-induced inflammation (see Tables XVII and XV and Figures 34 and 32, respectively).

Decato (55) demonstrated that the anti-inflammatory effects of cryogenine are similar to those of tetrabenzine which are in turn similar to those of chlorpromzaine, and associated with, but not necessarily dependent upon, the release of endogenous corticosteroids.

As stated earlier, Vinegar et al. (36) have indicated that carrageenin-induced edema progresses in two phases. The initial phase occurs about one hour after injection and the second phase, which is about three times as large as the first, occurs about

three hours after injection. The first phase is inhibited by aspirin, and is relatively insensitive to hydrocortisone and phenylbutazone. Our studies confirmed this. The second phase of carrageenin-induced edema is very sensitive to inhibition by the classical anti-inflammatory agents such as hydrocortisone, indomethacin, and phenylbutazone. Again, our study confirmed this. Our study also showed that cryogenine had a more extensive inhibiting effect upon the latter phase of edema formation. This data is in agreement with that of Decato (55).

In summary, all of the above drugs (with the exception of cryogenine) displayed a similar pattern of anti-inflammatory activity against velella-induced inflammation, possibly indicating that carrageenin and chitinous materials induce inflammation by a similar mechanism of action. Since cryogenine is a rare alkaloid, insufficient material was available to conduct further studies attempting to explain why this agent appeared to be an exception.

Further evidence should be gathered especially relative to determining the minimum molecule or molecules involved in initiating the inflammatory response with chitins, before this carrageenin-like hypothesis can assume the prestige of theory.

CONCLUSION

At present, the treatment of inflammatory diseases is in an unsatisfactory state. Progress is hampered not only by a lack of knowledge of the etiology of such diseases, but also by the lack of information on the many mediators of the inflammatory response. Development of therapeutic agents, therefore, is taking place against an incomplete theoretical background. Experimental animal models of the human disease are presently inadequate, and the drugs now used for treatment are by no means ideal. This situation obviously needs to be corrected, but where does research begin? The spectrum of uncertainty is so large.

These present experiments were undertaken in the hope of defining an alternative model of inflammation -- a unique system different from those utilizing either mycobacterium adjuvant or carrageenin.

Chitinous materials left by microorganisms were suggested as the possible cause of inflammatory disease and the following conclusions about invertebrate chitins were made utilizing animal studies:

(i) chitinous materials are phlogistic;

(ii) chitinous materials produce inflammation of less intensity, but of longer duration than that produced by carrageenin;

(iii) chitinous materials produce inflammation completely different in character than that produced by mycobacterium adjuvant;

(iv) the partly deacetylated and deproteinated forms of natural chitin are not as phlogistic as the natural chitin; and

(v) the responses of chitin-induced inflammation to drugs appear qualitatively similar to the responses seen with carrageenin-induced inflammation thereby suggesting similar mechanism(s) of action.

These results suggest a new method for investigating the mechanism(s) by which connective tissue diseases are initiated. By extending this study, the exact chemical species triggering chitin-induced inflammation may be determined, and new avenues for drug design may be opened up.

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ADDENDUM A

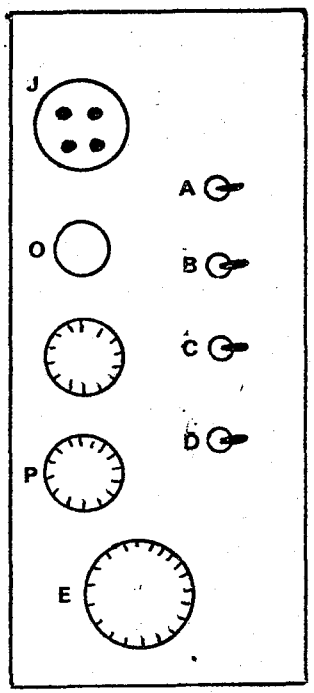
A STEPWISE CALIBRATION OF THE PLETHYSMOGRAPH

1. Flip toggle switch A to "arterial."
2. Flip toggle switch B to "negative."
3. Flip toggle switch C to "average."
4. Flip toggle switch D to "average."
5. Turn knob E to "1 mv./cm." (This is an approximate setting).
6. Turn knob F to "x.1 mv./cm." (This is an approximate setting).
7. Flip toggle switch G to "out."
8. Depress button H to "1 mm./sec."
9. Turn knob I to "operate" and allow at least 30 minutes, but preferably, 60 minutes for machine warm-up.
10. Adjust mercury level in S shaped tube with the mercury level in the reservoir using the syringe which is filled with distilled water.
11. Close the screw clamp which regulates the flow of water from the syringe which is filled with distilled water.
12. Flip toggle switch B to "off," turn knob N, and position pen $6\frac{1}{2}$ cm. from the top of the stylus paper.
13. Flip toggle switch B to "negative," turn knob O, and position pen $6\frac{1}{2}$ cm. from the top of the stylus paper.
14. Immerse calibration rod to the 1 ml. mark.
15. Turn knob O and again position pen $6\frac{1}{2}$ cm. from the top of the stylus paper.
16. Immerse the calibration rod to the 2 ml. mark. (This should position the pen 7 cm. from the top of the stylus paper. If not, adjust by turning knob P. Remember to release the lock on knob P before attempting to turn it!

17. Continue to immerse the calibration rod in 1 ml. increments, turning knob P slightly to keep the stylus pen positioned at 0.5 cm. intervals.

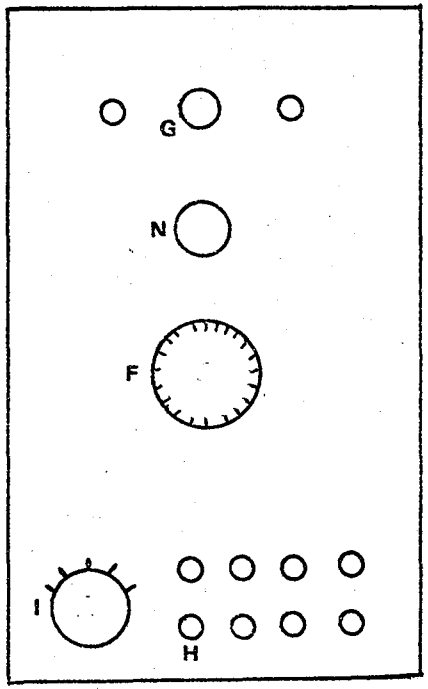
18. Withdraw the calibration rod. The pen should come to rest about 6 or $6\frac{1}{4}$ cm. from the top of the stylus paper.

19. Keep repeating steps 14 - 18 until linearity is achieved and each 1 ml. = 0.5 cm^2.



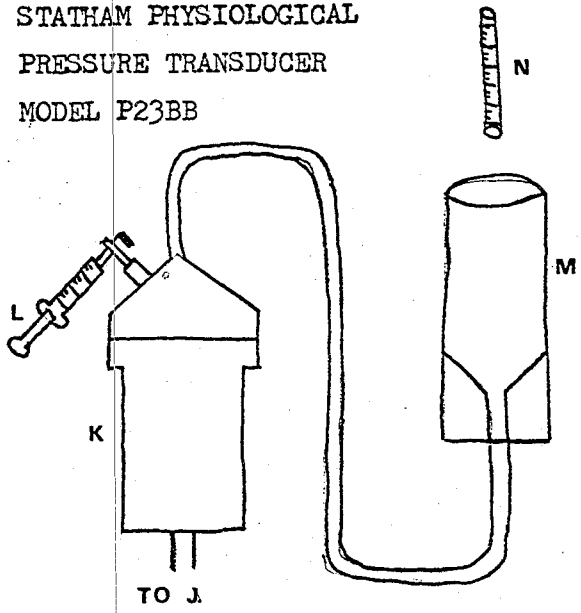
PRE-AMP.

BECKMAN
DYNOGRAPH
(TYPE RS)



AMP.

STATHAM PHYSIOLOGICAL
PRESSURE TRANSDUCER
MODEL P23BB



A - ARTERIAL/VENOUS SWITCH
B - +/- POLARITY SWITCH
C - NORMAL/ZERO SUPPRES SWITCH
D - AVERAGE/10mm Hg.H2O SWITCH
E - V/CM - MV/CM DIAL PRE-AMP.
F - V/CM - MV/CM DIAL AMP.
G - HI IN/OUT SWITCH
H - PAPER SPEED 1 MM/SEC.
I - OFF/OPERATE SWITCH
J - STRAIN GAUGE COUPLER
K - PRESSURE TRANSDUCER
L - SYRINGE
M - MERCURY RESERVOIR
N - PERSPEX ROD
O - BALANCE DIAL
P - TRANSDUCER SENSITIVITY DIAL