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Anticoagulant, Lipemia Clearing and Other Effects of Anionic Polysaccharides Extracted from Seaweed. (23528)

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Recent investigations have been made of the effects of heparin-like compounds on blood coagulation and lipemia clearance(1,2). This paper describes the hematologic and other responses to a number of such compounds. several of which have not been previously studied. These materials were prepared from aqueous extracts of red seaweeds. and are galactosans of varying degrees of sulphation.

Materials and methods. Carrageenan. a sulphated galactosan, and 6 other sulphated polysaccharides were studied.* Hyaluronate (3) and the chondroitin sulphates A(4) and B(5) were prepared using previously described methods. Measurements of lipemia were performed by reading directly the optical density of plasma in 12 x 70 mm cuvettes at 650 mμ in a Coleman Jr. spectrophotometer. Clotting times were determined in a series of small tubes ¼" in diameter containing 1 ml whole blood at 27°C. The tubes were inspected for coagulation every ½ minute. The endpoint was taken as the time when a tube could be placed on its side without any corresponding shift in blood level. Differential cell counts were done in triplicate. The hematoxylin-eosin and Hotchkiss-McManus periodic acid Schiff stains were done in the usual manner. Reducing activity was measured by a modification of the Park and Johnson procedure(6).

Twelve dogs were fed triolein (4 g/kg) after an overnight fast. The optical density of the plasma of all fasted animals was initially less than 0.09. Water was permitted *ad lib.* In 45 determinations on 12 dogs, all plasma turbidities two hours after feeding were within the range of 0.24 to 0.48 optical density unit (mean 0.41 ± 0.05). Immediately after removing 6-7 ml of blood 2 hours after the fat meal, the various polysaccharides prepared in a concentration of 2 mg/ml of saline were injected intravenously at a dose of 1 mg/kg body weight. Fifteen minutes after injection, a second blood sample was obtained. Blood was withdrawn in clean syringes and immediately added to tubes containing dry heparin. Samples of this blood were placed in clotting time tubes.

An aqueous solution of sodium heparinate was prepared containing 10 mg/ml and diluted with an equal volume of 0.3 N NaOH. After boiling this mixture for 15 minutes, the solution was cooled and 2 volumes of ethanol were added. The precipitate was removed by centrifugation and washed repeatedly with ethanol. This material demonstrated a threefold increase in reducing activity. A similar heparin solution was prepared using 6.0 N NaOH. The reducing activity increased some 15-fold and the molecule was partially desulphated. These materials were termed heparin A (normal), B (0.30 N NaOH), and C (6.0 N NaOH).

The seaweed extracts were purified by dissolving the commercial grade material in hot water and precipitating with isopropanol.

Results. The anticoagulant and lipemia clearance activities of the compounds described above were determined in triplicate.

* Seaplant Corp. of New Bedford, Mass., through the courtesy of Leonard Stoloff.

TABLE I. Effects of Some Anionic Polysaccharides on Lipemia Clearance and Clotting Time in the Dog.
(All figures represent means of measurements obtained in 3 dogs.)

Polysaccharide	Turbidity (O.D. at 650 mμ)	Clotting time (min.)	Comments
None	.30-.48	5-10	
Hyaluronate	.35	5	
Chondroitin sulphate A	.30	6.5	
B	.25	18	
Heparin A	.09	45	
B	.10	40	
C	.10	15	
Carrageenan	.44	5.5	dr* l
Extracts of:			
1) Furcellaria festigiate	.38	5.5	dr l
2) Eucheuma spinosum	.39	.12	de
3) *Gigertina acicularis*	.40	50	de
4) " *pistillate*	.35	6	de
5) " *radula*	.36	6	de
6) Iridaca	.40	5	de

* dr = drowsiness, l = lethargy, de = defecation.

Results are given in Table I. The range of response within any given trio of dogs was small. All dogs given the seaweed polysaccharides displayed yawning and lethargy, and fell asleep for about 10 minutes immediately after injection. Most of these animals also defecated immediately. The lethargy response occurred in over a dozen different dogs, while the anticoagulant activity of extract #3 (*Gigertina acicularis*) was not found in 2 of this group of 12 animals.

Unlike heparin, the anticoagulant activity of extract #3 increased with time, and was still evident 24 hours after injection. This change in activity with time was studied in 3 animals given extract #3 (1 mg/kg). Differential cell counts were determined in duplicate on each animal as well as the hematocrit and clotting time at ½, 1, 2, 4, and 24 hours after injection. During this time there were no changes in rectal temperature nor was any evidence of hemolysis observed. The anticoagulant activity was maximal after 4 hours, while none of the changes in the differential cell count or hematocrit were significantly different from control levels.

Three dogs were injected with 15 mg/kg of this polysaccharide (from *Gigertina acicularis*). Arterial and venous pressures and electrocardiograms of 2 of these were continuously recorded. The small changes that occurred were similar to those produced by equivalent amounts of 5% gelatin solutions. Both dogs died within 24 hours. The survivor was sacrificed at the same time. The tissues of these animals were fixed in formalin, sectioned and stained in duplicate with H. & E. and PAS.

Microscopically, all organs showed changes of venous congestion. The macrophages of the spleen and liver were strongly stained by PAS, indicating a powerful affinity of the polysaccharide for the reticulo-endothelial system.

The most remarkable changes were evident in the kidneys. The glomerular tufts had taken on the appearance seen in membranous glomerulonephritis. The basement membrane was strikingly thickened, and the glomerular epithelium showed vacuolation.

Discussion. These experiments were designed using a large amount of triolein (olive oil) to produce marked plasma turbidity. Under these conditions lipemia clearance will only occur when a very active compound is used. The alkaline degradation products of heparin were quite active in this respect, although the more degraded material lost its anticoagulation activity. The observation has been reported by Zollner and co-workers (7).

Extract #3 is a very potent anticoagulant. The mechanism of this anticoagulant activity is currently under study. The profound pathological changes in the kidney that occur with large doses of the polysaccharide give the appearance of being a direct reaction with some component of glomerular membrane or an extreme local alteration in osmotic equilibrium. Popper *et al.*(8) have shown that intravenous pectin infusions caused a dilation of the renal tubules and glomerular spaces, as well as a deposition of material that resembles amyloidosis. These effects were also seen with extract #3. The renal function of animals so treated is under study.

Summary. A number of anionic polysaccharides were investigated with respect to their anticoagulant and lipemia clearing activity. Only heparin was effective in clearing

the massive lipemia used in this study. This effect persisted even when the anticoagulant activity was destroyed by alkaline degradation. Another potent anticoagulant was found in a highly sulphated galactosan isolated from seaweed. This compound produced extensive pathologic changes in the kidney when administered in large doses.

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Effect of Some Monosaccharides on Serum Inorganic Phosphate in the Normal Dog. (23529)

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Soon after the discovery of insulin, it was found that the hormone has a depressing effect upon serum inorganic phosphate(1). A similar effect was described after glucose administration(2,3). The action of glucose on serum inorganic phosphate appeared to be due to induced insulin secretion since it was not possible to elicit a normal response in the pancreatectomized dog(4), or in the alloxanized dog(5). It seemed of interest to study the effect of various monosaccharides upon serum inorganic phosphate. It has been mentioned that glucose, fructose and galactose show a depressing effect(6,7). It has been also demonstrated that fructose may decrease serum phosphate in the pancreatectomized dog (8). In this paper, the effects of 8 sugars and one polyalcohol (D-Sorbitol), independently injected by vein and in some cases mixed with glucose and/or insulin are reported.

Material and methods. Apparently normal dogs weighing between 12 and 20 kg, fed *ad libitum* on Purina Chow and twice weekly on meat, were used. The animals were previously trained by repeated venipunctures to avoid fear and anger reactions. When the same dog was used for several experiments, a previous test was carried out on a different day with glucose in order to check whether the animal showed a normal phosphate fall. The experiments were carried out after a fasting period of about 15 hours. A venous blood sample was taken, the sugar injected in 3M solution (1 ml/kg) within a period of one minute and new samples obtained at 15, 30, 45 and 60 minutes. D-Galactose was used in 1.5M concentration (2 ml/kg). When 2 sugars were administered, one ml of the 3M solution/kg of each was injected. Glucagon-free insulin (generously furnished by Eli Lilly Co.) was used by vein at the dose of 0.1 U/kg. Serum inorganic phosphate was determined in duplicate using the Fiske and Subbarow procedure(9) adapted to the photoelectric colorimeter. Changes in serum inorganic phosphate were expressed in terms of "Deltas" (differences from the initial values in mg/100 ml). The statistical significance of the results was appraised with the use of the "t" test, by comparison with the spontaneous variation observed by taking blood samples every 15' up to 60', under experimental conditions. The compounds used (C.P.) were:

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