EXPERIMENTAL STUDIES OF ALGINATES AS HEMOSTATICS*

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THE THREE ABSORBABLE hemostatic packing materials which have had extended trial in clinical surgery are, in order of their introduction, human fibrin foam, oxidized cellulose—in the form of gauze or cotton—and gelatin sponge. There are minor differences in the immediate hemostatic effect of these different materials and in the technics of their surgical use, but they are all non-irritating in the tissue, non-toxic and absorbable. None of these can be sterilized by autoclave, and should a material be developed which had this advantage and also the desirable properties of the other agents it would be a welcome addition to our surgical armamentarium.

It was suggested that alginic acid or its salts might answer this purpose. Alginic acid is a naturally occurring organic acid derived from seaweed (Laminaria digitata) with a formula closely resembling cellulose.[1] The alginates had already been developed for wide commercial usage. Woven and spun preparations, gauze and stockinette were available. As is often the case with what is thought to be an original idea, independent investigations were undertaken in several different laboratories. The credit for the first experimental observations goes to Blaine, of England, whose paper, although not published until January 1947, was submitted to this journal for publication in January 1946.[1]

Our own studies began about that time and we were accelerated because we had the privilege of reading Major Blaine's article in manuscript. As he says in his text, "Exigencies of the service in wartime have made it impossible to conduct a more complete examination of the many problems at issue. It has been shown, however, that alginates possess certain properties which make their surgical use attractive." There was no doubt in our minds, therefore, that the alginates held considerable promise. We had hoped to obtain directly from Major Blaine some of the alginate gauze he had employed in his studies for comparison with that submitted to us by the chemists who had suggested its possible surgical application, comparison particularly in regard to texture and tensile strength. We also planned to run a comparative series of experiments on oxidized cellulose and this new material, with especial reference to the immediate effective hemostatic properties of each agent, and the tissue reactions. Somewhat later it seemed imperative that the pharmacology of the alginates should be studied in detail for comparison with oxidized cellulose and this was undertaken by another group of investigators.[2] Stimulus was also given to this study by the publication of a letter to the editor in Science, May 1946, in which Smith[3] reported the styptic action of local application of

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alginic acid powder in 100 cases of tooth extraction and minor oral surgery. The dose would obviously be small in these cases and presumably most of the wounds were open.

The alginates submitted to us by the Research Laboratories of Johnson and Johnson were processed in an effort to maintain tensile strength and achieve ease of handling in actual surgical manipulation. Early samples of varying calcium content were submitted, and in addition, a sizing agent as well as a buffering solution were sometimes used. The chemical analysis shown in Table I makes it clear that variations in tissue reaction could therefore be attributed to a number of factors.*

With all samples it was observed that the tensile strength was not as good as that of oxidized cellulose, and that the combination of the material with citrated blood resulted in a soft, brownish-black, mushy paste more difficult to handle with forceps than the black gelatinous mass so formed with oxidized cellulose. It was expected, however, that if other features were satisfactory, minor changes in preparation might alter these physical properties. The

* METHOD OF PREPARATION

Calcium Alginate Gauze:

31B, 35A, 35C	—	Treatment: 15 minutes in HCL, pH 1.5. Washed, buffered, 15 minutes, washed, air-dried.

100 (4) Denier Calcium Alginate Stockinette:

62D1, 69	—	Treatment: Washed in water. Extracted with ethyl alcohol and ether (separately), treated in HCL (0.974N) 1 hour, washed and treated 2 hours in an excess of 0.2N $CaCL_2$, washed. Buffered, air-dried.
73		Treatment: Similar to 62D1 and 69 but not buffered. Washed with tap water, pH 7.4, dried at 45°.
62B1, 65	—	Treatment: Washed in water. Extracted as 62D1 and 69, treated overnight in 0.974 N HCL, and washed CL free. Buffered, washed, air-dried.
64	—	Treatment: Approximately normal HCL 1 hour. Washed once and treated in an equal quantity of fresh acid 30 minutes. Washed. Buffered 20 minutes. Washed, air-dried.
41-116	—	Treatment: Dilute HCL until calcium content reduced to 0.15%. Buffered.
41-118	—	Treatment: Similar to 41-116 but alcohol-ether extracted to remove Fixanol.
41-120	—	Treatment: Warm water (40° C.) Calcium content reduced with dilute HCL (3% then 1%). Washed with cold water. Dried at 60°.
41 151	—	Treatment: Extracted with acetic acid to remove Fixanol. Dilute HCL to remove calcium. Alginic acid thus derived oxidized with HIO_4, with partial conversion of the secondary alcohol groups to aldehyde groups. Treated with dilute HNO_3, air-dried.
41-152	—	Treatment: Similar to 41-151 but Fixanol not extracted.
89-31, 89-32	—	Treatment: Fuming HNO_3 at room conditions 1 hour. Washed free of acid, air-dried.

affinity of the alginic acid for hemoglobin as demonstrated by the laked blood test was similar to that of oxidized cellulose.

In our earlier studies of oxidized cellulose[4, 5] one of the standard tests was solubility in 0.15 Molar solution of sodium bicarbonate. Satisfactory preparations of oxidized gauze and oxidized cotton dissolve completely without residue. No animal tests were undertaken on samples which showed re-

TABLE I.—*Alginates Tested*

Lot No.	Weave	% Free COOH	% Ca	Buffered* %K	Nitrated %N	Fixanol† Sizing	Sterilization
31B	Gauze	22.14	3.03	Approx. 2.5	..	+	Steam
35A	"	"	2.45	"	..	+	"
35C	"	"	3.06	"	..	+	"
62D1	Stockinette	"	2.80	"	..	0	"
69	"	"	1.82	"	..	0	"
73	"	"	2.81	0	..	0	"
62B1	"	"	0.25	"	..	0	"
65	"	"	0.58	"	..	0	"
64	"	"	0.19	"	..	+	"
41-116	"	"	0.10	Analyzed 2.54	..	2%	"
41-118	"	"	0.10	"	..	0	"
41-120	"	"	0.10	Approx. 2.5	..	+	"
41-151	"	"	0.0	..	0.2	0	Formaldehyde 0.11 res.
41-152	"	"	"	..	0.3	2%	"
89-31	"	16.3	"	0.0	4.0	Trace	Formaldehyde
89-32	"	14.6	"	"	4.68	"	"

*Potassium acid phthalate solution 0.05 Molar pH 4.0
†Sizing: Fixanol—octyl pyridium bromide. A finishing agent used in the manufacture of alginate yarn to prevent sticking together of the monofilaments.

TABLE II.—*Standard Rat Tests on Calcium Alginate Gauze*

Lot No.	% of Ca Content	No. of Animals	Days Post-Op.	Absorbed	Inflammation	Fibrosis	Conclusions
31B	3.03	5	2—28	0	2+	1+	Unsatis.
35A	2.45	5	7—21	0	1+	1+	Unsatis.
35C	3.06	5	7—21	0	2+	1+	Unsatis.
62dl	2.80	4	4&7	0—±	Contam.	Contam.	Repeat*
69	1.82	6	2—7	±	1+	2+	Unsatis.
73	2.81	5	2—7	±	±	±	*Also unsatis.
62Bl	0.25	6	7	Completely	0	0	Satis.
64	0.19	6	2	Completely	Min.	..	Satis.
65	0.58	4	2	Completely	Min.	..	Satis.

sidual fibers of material. In applying this test to calcium alginate it also was found to be soluble in 0.15 Molar $NaHCO_3$, but there was a precipitate of calcium carbonate which was not to be confused with insoluble residue. All samples tested were completely soluble.

Affinity for hemoglobin, and solubility tests having borne out the expectation that alginic and cellulosic acid were somewhat similar in behaviour as

well as structure, tests for tissue reaction were then undertaken. The first tests were for absorption and tissue response and were conducted according to the technic devised by Lattes Frantz.[6] This was reported in this Journal in 1945. It consists of aseptic implantation of materials under scrutiny in the subcutaneous tissues of the back of rats. In our own previous studies, fibrin foam, gelatin sponge, oxidized cellulose and a large number of other substances had been subjected to this "rat test." Only the first three were found to be

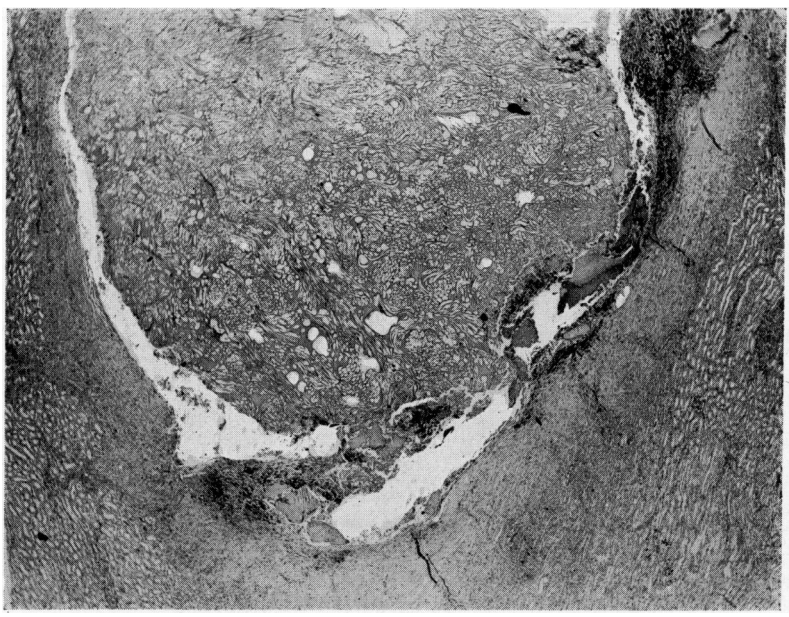

FIG. 1.—S. P. 25509. Photomicrograph, X38, of di-aldehyde alginic acid gauze (41-151) in experimental wound of upper pole of kidney. Dog —42 days. Large amount of unabsorbed material. A broad zone of fibrous scar tissue separates kidney parenchyma from the hemostatic packing. On higher magnification this tissue is seen to contain many hemosiderin laden phagocytes. There are no polymorphonuclear leukocytes and no foreign body giant cells.

both non-irritating and completely absorbed. In testing the alginic acid preparations by this method it was thought unnecessary to run simultaneous controls of oxidized cellulose since detailed studies had already been reported (4 and 5) and the techniques used were the same. The results of the first series of rat tests are shown in Table II.

These early promising samples (62B1, 65 and 64) led us to study the effectiveness of the new materials in experimental hemorrhage. It will be seen from Table II that the unsatisfactory samples were those of higher calcium content, and the trials of hemostasis were accordingly made on approximate duplicates of 62B1, but with even lower calcium content (41-116, 41-118 and 41-120). Again the tests employed were those by which oxidized cellulose had originally been studied and which have been reported in detail (4

and 5), i.e. wounds of kidney and spleen. Immediate hemostatic effect in an experimental or clinical wound is obviously not subject to exact measurement. The wounds of the kidney in this technique are made as nearly comparable as possible, one in the upper and one in the lower pole. The capsule is incised, a clamp is thrust into the parenchyma and spread so that brisk bleeding ensues. The wound is then lightly packed with the hemostatic agent to be studied. It was in such wounds that we first observed that oxidized gauze

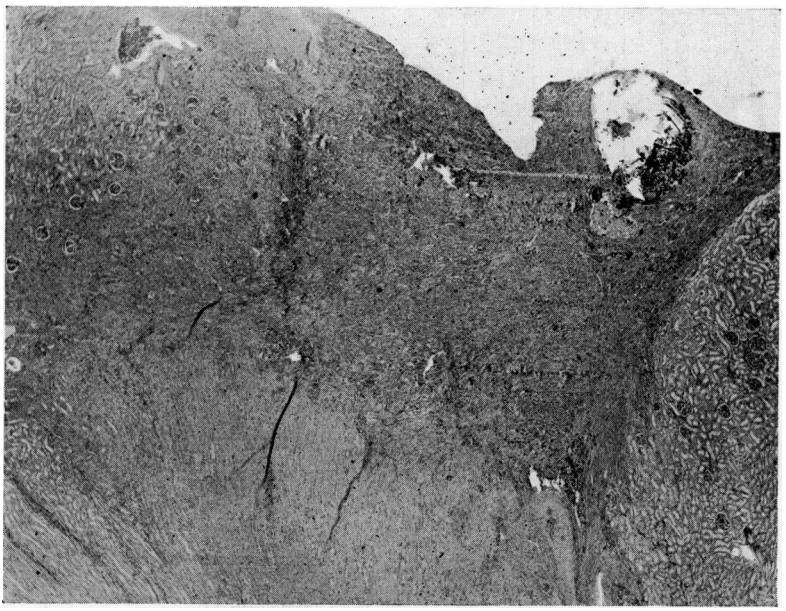

FIG. 2.—S. P. 25509. Photomicrograph, X38, of control mound, lower pole of same kidney as Fig 1, packed with oxidized cellulose. The foreign body in the capsule is the markingsilk suture. No gauze is seen. The site of the implant at this magnification is characterized by diffuse dark staining. There isc no unabsorbed material. In higher magnification this zone is almost entirely composed of phagocytes, some hemosiderin laden. The bulk show the basophilic staining characteristic of cellular reaction to oxidized cellulose. There is almost no scar tissue except the linear extension of the wound seen at the base on the right, and slight fibrous prolif eration in the capsule.

had a specific hemostatic effect. Hemorrhage was more rapidly controlled with this gauze than with plain gauze. The alginate preparations when compared with oxidized cellulose exhibited very little hemostatic effect. The hemorrhage was controlled in these standard kidney wounds chiefly by the presence of packing and not by any evident styptic action.

Further confirmation of this was obtained in standard tests in shallow surface defects in the spleen where the packing effect is absent. Here little styptic action of alginate was noted, although the preparation without sizing was somewhat more effective than those with it. Controls here were also made with oxidized gauze. (Table III).

TABLE III.—*Comparative Studies in Experimental Hemostasis and Tissue Reactions in Dogs with Oxidized Cellulose and Alginic Acid*

	Lot No.	Chemical Analysis	Autopsy Days Post-Op.	Immediate Hemostatic Effect in Spleen and Kidney		Tissue Reactions in Kidney: Absorption		Tissue Reactions in Kidney: Evidence of Inflammation		Comment
				Alg.	Ox. Cell.	Alg.	Ox. Cell.	Alg.	Ox. Cell.	
1.	41–116 (Chenoweth B)	Buffered Sizing Ca 0.1%	17	1+	4+	3+	4+	3+	0	Progressive downhill course.
2.	41–116	"	40	±	4+	2+	4+	3+	0	Sick for 6 days post-op.
3.	41–118	Buffered. No sizing Ca 0.1%	4	2+	4+	0	0	3+	3+	Peritonitis. No culture.
4.	41–118	"	28	2+	4+	2+	4+	1+	0	Sick for 5 days post-op.
5.	41–120	Not buffered No sizing Ca 0.1%	28	±	4+	4+	4+	3+	0	Very sick for 5 days post-op.
6.	41–120	"	40	±	4+	2+	4+	3+	0	Loss of weight and strength throughout
7.	41–151 (Chenoweth C)	Di-aldehyde No sizing Ca 0%	42	2+	4+	2+ (Fig. 1)	4+ (Fig. 2)	1+	0	Poor health throughout post-op course.
8.	41–152 (Chenoweth C)	Di-aldehyde Sizing Ca 0%	7	±	4+	±	±	2+	1+	Died. Antemortem clot, rt. auricle.

As the immediate hemostatic effect of the alginates did not compare favorably with that of oxidized cellulose, further chemical modifications of the preparations was proposed in order to improve this property. One of these new preparations was the di-aldehyde of alginic acid (41-151 and 41-152) and this was moderately effective in immediate hemostasis. (Table III). It was not a promising modification, however, because the material, originally proposed to facilitate sterilization, no longer had the advantage of being sufficiently heat resistant to be sterilized by autoclave. Another preparation suggested, nitrated sodium alginate, was also too unstable for autoclave. In spite

TABLE IV.—*Standard Rat Tests on Recent Preparations*

Lot No.	Description	No. of Animals	Days Post-Op.	Absorbed	Inflammation	Fibrosis	Conclusion
41-120	Alginic acid. Not buffered. No sizing Ca 0.1%	5	2—8	Completely	Variable	1+	Unsatis.
41-151 (Chenoweth C)	Di-aldehyde No sizing Ca 0%	5	2	Completely	0	0	Satis.
41-152 (Chenoweth C)	Di-aldehyde Sizing Ca 0%	5	2	Completely	0	0	Satis.
89-31 (Chenoweth D)	Nitrated sodium alginate with sizing	4	1—7	Completely	1+	0	Toxic*
89-32 (Chenoweth D)	Nitrated sodium alginate with sizing	4	2—5	Completely	±	0	Toxic†

*1 Death—24 hours. 2 Animals lethargic until sacrificed.
†3 Deaths—2, 2 and 5 days. 1 Animal lethargic when sacrificed at 3 days.

of this disadvantage a second short series of rat tests was undertaken as a preliminary to possible further studies on hemostasis. The results are shown in Table IV. At the time these tests were made we were apprised of the observations of Chenoweth on the toxicity of the material in cats.[2] Detailed autopsy studies were not attempted on the rats in this last series because postmortem changes were often advanced, and there were further depredations by cage mates. In comparison, however, with several hundred previous rat tests the morbidity and mortality rate in this group was considered significant.

With this knowledge, a careful re-reading of Blaine's article led us to the belief that although the cats he subjected to liver lacerations had survived when the bleeding had been controlled with alginates, there had been early toxic effects comparable to those reported by Chenoweth. Moreover, the polymorphonuclear leukocytic response which the author described in the local tissue reactions, had been duplicated in many of our own observations, and indicated a greater degree of irritation caused by the alginates than by oxidized cellulose.

SUMMARY AND CONCLUSIONS

1. Local tissue reaction to various spun and woven preparations of alginic acid and its derivatives have been studied in a series of rat tests. Although some of the preparations evoked a minimal inflammatory response, several were apparently irritating and engendered a considerable fibrous tissue replacement when finally absorbed. The most favorable preparations were slightly more irritating than oxidized cellulose. That they were also toxic even when introduced thus, in solid form, to be absorbed slowly, was suggested by the high mortality rate among these rats, although detailed study of the toxicology was not undertaken here.

2. The immediate hemostatic effect of alginic acid preparations was contrasted with controls of oxidized gauze in the spleen and liver of dogs. There was a marked difference in this property, control of hemorrhage being often difficult to obtain with alginate when oxidized cellulose was immediately effective. Study of these organs at autopsy showed in general, more inflammatory and fibrous tissue response to the alginates than to oxidized cellulose. If anything, the absorption time of the former was longer, but this was not checked in detail. The toxic effects at presumably lower dose levels than those employed by Chenoweth in cats suggests the possibility that the dog is more susceptible to the toxic effects of the alginates than the cat.

3. It was concluded that the advantage of sterilization by autoclave was outweighed by the less effective hemostatic action and by the toxic properties of the alginates, and that further experimental study was not warranted. It was not thought safe to propose these new preparations for trial in clinical surgery.

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