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Oxidized Cellulose and Thrombin.

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The use of absorbable cellulose[1,2,3] as a vehicle for thrombin to control hemorrhage offers new possibilities for securing effective hemostatis.[4]

We have observed that some combinations of thrombin and oxidized cellulose are damaging to thrombic activity. Further investigation has shown that there are two factors which can cause the inactivation. One is the inherent acidity of the oxidized cellulose, and the other is an unidentified product of the oxidation process. In order to demonstrate these facts it is necessary to vary the oxidation of the cellulose, in a manner which will separate the two factors which contribute to thrombin denaturation.

The oxidation of cotton with nitrogen dioxide gas has been described by Yackel and Kenyon,[1] and by Unruh and Kenyon.[2] These authors have also shown that the carboxyl content of the material increases as oxidation progresses. The carboxyl content can be measured by a simple titration, and by that means the progress of oxidation can be followed. We were supplied with oxidized cotton from the laboratory of the authors mentioned above, and had an opportunity to study its effect on thrombin.

Material with a carboxyl content of 14% was first studied. Approximately 100 mg of the cotton was mixed with 5 cc of bovine thrombin prepared by the method of Seegers.[5] The pH of the solution, measured with a glass electrode, was found to be below 4.3, which is the inactivation point for thrombin. With the use of standardized fibrinogen[6] the thrombin activity of the solution was quickly measured several times during the first 15 minutes after mixing, and was found to be disappearing very rapidly (Fig. 1).

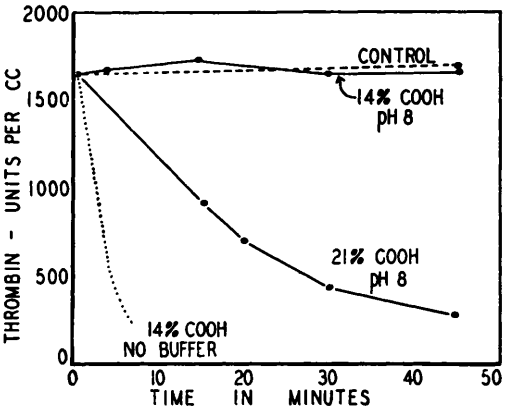

FIG. 1.

Mixtures of oxidized cellulose and thrombin are shown to be stable at pH 8 provided oxidation of the cellulose is not excessive. Without buffers the rate of thrombin inactivation varies markedly with the relative quantity of cellulose mixed with the thrombin.

In order to show that such loss of activity is due only to the acidity of the cotton, the same experiment was repeated with only one change in technic. The cotton was first soaked in 10 cc of 1% $NaHCO_3$ solution for 3 minutes, roughly dried between filter papers, and mixed with 5 cc of thrombin solution. The latter remained alkaline (pH, 8) and retained its original activity in full.

When oxidation of the cellulose is more extensive than required for rapid *in vivo* absorption by tissues, acidity continues to be a factor, but in addition other products of oxidation destroy thrombin. The inactivation process is slow, but eventually all active protein is destroyed. Fig. 1 shows the decline in activity, expressed in quantitative units,[6] when the carboxyl content is 21%. The cotton (100 mg) was soaked in 10 cc of 1% $NaHCO_3$ solution for 3 minutes, and then blotted dry between filter papers. When this

1 Yackel, E. C., and Kenyon, W. O., *J. Am. Chem. Soc.*, 1942, **64**, 121.

2 Unruh, C. C., and Kenyon, W. O., *J. Am. Chem. Soc.*, 1942, **64**, 127.

3 Frantz, V. K., *Ann. Surg.*, 1943, **118**, 116.

4 Putnam, T. J., *Ann. Surg.*, 1943, **118**, 127.

5 Seegers, W. H., *J. Biol. Chem.*, 1940, **136**, 103.

6 Seegers, W. H., and Smith, H. P., *Am. J. Physiol.*, 1942, **137**, 348.

bicarbonate treated material was placed in 5 cc of thrombin solution the resulting pH was 8; but the thrombin activity disappeared even though the acidity had been controlled properly. The nature of the destructive factor(s) is not known.

Summary. Oxidized cellulose injures thrombin due to its acidity. After neutralization with sodium bicarbonate it can be mixed with thrombin without altering activity. Highly oxidized cellulose contains products capable of destroying thrombin even in neutral solutions.

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Influence of Anesthesia on Circulatory Changes in Dogs Subjected to Graded Hemorrhage.*

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One of the chief difficulties in a study of the shock syndrome in dogs has been the lack of a critical evaluation of the effects introduced by the anesthetic agent. This paper deals with an analysis of the effects of several anesthetic procedures on the circulatory changes in the dog resulting from a standardized bleeding procedure. By using the changes in the omental circulation as an index it was found that criteria, previously established,[1,2] served as a means of differentiating between specific effects of different anesthetic agents.

Method. Fifty-three dogs were anesthetized with the 6 different drugs indicated in Table I: (1) procaine—local anesthesia, (2) morphine sulphate, (3) cyclopropane, (4) ether, (5) sodium pentobarbital, (6) sodium pentothal. Before anesthesia was induced the femoral artery was cannulated during local procaine infiltration (2 cc of 1% solution). Blood pressure were recorded continuously. Following induction of anesthesia the omentum was exteriorized for direct microscopic study according to a method previously described.[3] In Group 1, (Table I), which served as controls, pain relief for the required surgery was obtained with an abdominal field block using 8-10 cc of a 1% procaine solution. Group 2 was sedated with morphine sulphate given intravenously (2 mg/kg). Groups 3 and 4 were anesthetized with cyclopropane and ether respectively, administered with oxygen. The endotracheal to and fro absorption technic was employed. They were maintained unconscious, in upper first plane surgical anesthesia throughout.[4] Group 5 received a single intravenous dose of 25-30 mg/kg of sodium pentobarbital. Oxygen by endotracheal catheter was given to 3 dogs in this group. As no demonstrable differences were noted in the dogs with or without oxygen in this group, the results are considered together. Group 6 received sodium pentothal (1% solution) intermittently by vein to maintain a similar degree of narcosis and required an average of 10.8 mg/kg/hr. All of this group were given oxygen. Hemorrhage in each instance was accomplished with an initial blood-loss of 2% body weight and continued by removing an additional 0.5% at successive 30-minute intervals. To standardize the procedure, the maximal blood-loss compatible with viability was considered to be that which brought about a cessation of blood-flow in the larger omental arteries under observation during the bleeding. The majority of the dogs lived for at least 60

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4 Guedel, A. E., *Inhalation Anesthesia*, Macmillan and Company, 1937.