THE ANTIGENICITY OF BOVINE THROMBIN:
CLINICAL EVALUATION

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The recent rise to importance of thrombin as a hemostatic agent has precipitated a real though largely unpublished debate on the subject of its antigenicity. Two incidental and unrelated factors have tended to promote division of opinion. On the one hand the tremendously rapid strides made in the purification of beef thrombin by the Iowa group under the leadership of Seegers, followed by proofs of its usefulness in surgery, have outdistanced research in the subsidiary problem of antigenicity. On the other, the collection of large quantities of human blood by the Red Cross for the benefit of the combat troops has afforded, in connection with its conversion to serum albumin, an abundant though temporary supply of human thrombin, the superiority of which has been championed by the Harvard group which sponsored the program of blood fractionation. The gulf between those who hold beef thrombin harmless in its surgical application and those who imply danger in the use of thrombin from any source except human blood remains unbridged by a connected line of evidence, and at best has been only sampled.

Antigenicity is a titanic problem handicapped by an interdiction against free transfer of animal evidence into human conclusions, and by a wide personal choice of values ranging from the scientifically absolute to the clinically tolerable. In this paper I shall do no more than to marshall the available evidence, add a new sampling, and reach an obviously temporary opinion. The question of the safety of animal thrombin, however, is of the moment: the cancellation in large part of the production of serum albumin resulted unavoidably in reduction of the by-products from which up to now human thrombin has been derived; the philanthropic nature of the blood collections by the Red Cross prevented the distribution by sale to civilian users; and surgeons therefore are giving increasing acceptance to the clotting agents derived from animal sources, such as bovine thrombin and rabbit clotting globulin, which have now been released by the National Institute of Health for commercial distribution. The case against bovine thrombin will be considered first.

POSITIVE RESULTS IN THE EXPERIMENTAL FIELD

1. EXPERIMENTS OF DR. BEATRICE CARRIER SEEGER

There are no reports in the literature indicating that sensitization has been produced by injections of bovine thrombin, but immunologists affirm generally that thrombin, being a protein, under favorable experimental con-
conditions should provoke antigenicity of some degree. A series of experiments done by Dr. Beatrice Carrier Seegal yielded successful sensitization of guinea pigs and rabbits to bovine thrombin. She has kindly communicated to me her experiments which may be summarized as follows:

Sensitization of Guinea Pigs. Eleven guinea pigs were injected subcutaneously with 0.5 or 1.0 mg. of alum precipitated bovine thrombin. Four weeks later, 6 of these animals were injected intravenously with 0.6 or 1.0 mg. of the thrombin in solution, given slowly over a period of 30 to 60 seconds in the foot vein. All 6 animals died within 3 to 4 minutes, showing the respiratory difficulty, the cyanosis, and the edema of the lungs typical of anaphylactic shock. The remaining 5 animals, after reactionless injections with human thrombin, likewise succumbed when bovine thrombin was injected. In addition to the 11 animals thus sensitized by subcutaneous injection of an alum precipitated bovine thrombin, 3 animals (weight 350 to 400 gm.) were sensitized by intravenous injection of 1.4 to 2.0 mg. of thrombin in solution and tested for sensitivity 4 weeks later by the intravenous injection of 1.0 mg. of the antigen. Two of these animals died of anaphylactic shock, and the third showed symptoms of moderate shock with a drop in temperature of 1.8°F.

Sensitization of Rabbits. Three rabbits were injected intravenously with a total of 9 mg. of each of alum precipitated bovine thrombin over a period of 24 days. Six days after the last injection a sample of blood was drawn from each animal. The serums were tested for precipitins with two lots of bovine thrombin and one lot of human thrombin, diluted 1–500 and 1–5000. All 3 serums strongly precipitated with both dilutions of the bovine thrombins. A trace of precipitate occurred between human thrombin 1–500 and the serum of one rabbit. On the 7th day, the 3 sensitized rabbits were injected into the left cerebral area with bovine thrombin in amounts of 0.6, 0.4 and 0.4 mg. respectively in 0.3, 0.2 and 0.2 cc. physiological saline. Three normal rabbits were each similarly injected with 0.6 mg. of bovine thrombin in 0.3 cc. saline. The sensitized animal receiving the larger dose and 1 normal rabbit, 1½ to 2½ hours later, began circling to the right and falling to the right side, and both animals were dead the following morning. The other two sensitized animals showed clonic right-sided movements (and less active twitching on the left) beginning the day following injection and persisting until sacrifice at 3 days. At autopsy both of the rabbits that died at 20 hours showed slight gross increase in the size of the left cerebrum. One sensitized animal showed a centrally located area of hemorrhage and softening about 7 mm. in diameter, and this proved microscopically to contain nerve cells undergoing ischaemic necrosis, in addition to hemorrhage, oedema, and leucocytic infiltration—the “Arthus phenomenon.”

A second type of rabbit experiment was done by replacing the anterior chamber fluid of the eye with 0.4 mg. bovine thrombin dissolved in 0.2 cc. physiological saline. Four weeks later an intravenous injection of 3.5 to 4.0 mg. of bovine thrombin in solution brought death from anaphylactic shock to 1 out of 7 animals thus tested.

Important as these experiments are, certain conditions should be noted: a) The amount of material injected to produce shock was one-quarter to one-half the amount that had produced reactions in unsensitized animals; for example, a normal 208-gm. guinea pig died ten minutes after the rapid injection of 2.0 mg., while the remaining control animals, receiving 1.4 to 2.0 mg. by slow intravenous injection, showed temporary respiratory increase following injection. Likewise, a rabbit receiving 3.5 to 4.0 mg. of thrombin intravenously in the course of 4 minutes, died of intravascular clotting, and the injection time had to be prolonged to 10 to 20 minutes to permit survival by the rest. b) The potency of the solution of thrombin used intravenously is given as 100 Iowa units per mg., but the strength of the alum precipitated