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Quantitative Studies on Antithrombin.*

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The normally existing anticoagulants of blood serum and plasma according to present concepts are antiprothrombin and antithrombin. Howell[1] stated that blood normally contains but a small amount of antithrombin. A critical analysis of previous methods for the quantitative determination of antithrombin has only become possible with the purification and standardization of prothrombin and thrombin by Warner, Brinkhous, Smith and Seegers.[2] Previous methods have consisted of either adding considerably less than one unit of thrombin to undiluted serum and observing the clotting time upon the subsequent addition of fibrinogen after various periods of incubation[3, 4, 5] or by adding a small fraction or a few units of thrombin to plasma and observing the variations in the clotting time.[6, 7] The obvious conclusion was that serum or plasma can inactivate or neutralize very little thrombin. It is well established that hemorrhage may occur with plasma prothrombin levels of 35% of normal (105 units) or less. Theoretically there should be a marked excess of prothrombin even in this hemorrhagic zone inasmuch as one unit of prothrombin when converted to thrombin will convert 1 cc of fibrinogen to fibrin in 15 seconds. From these observations it would seem probable that the antithrombic activity of serum and plasma is much greater than previously assumed.

Before a method was devised for the quantitation of antithrombic activity of serum or plasma, the quantitative adsorption of thrombin was observed during the conversion of fibrinogen to fibrin. To various amounts of purified fibrinogen were added 60 units of

* Aided by a grant from the Comly Research Fund, Ohio State University.

1 Howell, W. H., *Physiol. Rev.*, 1935, **15**, 435.

2 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667; Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801; Seegers, W. H., Brinkhous, K. M., Smith, H. P., and Warner, E. D., *J. Biol. Chem.*, 1938, **126**, 91.

3 Howell, W. H., *Arch. Int. Med.*, 1914, **13**, 76.

4 Gasser, H. S., *Am. J. Physiol.*, 1916, **42**, 378.

5 Mills, C. A., and Kitzmiller, K. V., *Arch. Int. Med.*, 1926, **38**, 544; *ibid.*, 1927, **39**, 618.

6 Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.

7 Eagle, H., *Bull. Johns Hopkins Hosp.*, 1937, **60**, 428.

purified thrombin and the amount that disappeared from solution calculated. The conversion of 1 mg of fibrinogen resulted in the adsorption of 5.1 units of thrombin upon the fibrin strands; no further adsorption occurred regardless of the time of incubation in the presence of excess amounts of thrombin. Therefore when blood coagulates, approximately 90% of the thrombin must be inactivated or neutralized by the normal antithrombic activity of the serum.

Observations were then made on the quantitative activity of the antithrombin of diluted and undiluted serum and oxalated plasma. The fibrinogen was removed from plasma by incubation for 10 minutes at 56°C. When 1 unit of thrombin was added to 1 cc of various dilutions of serum and plasma and the amount of thrombin calculated that was inactivated at various periods of incubation at 28°C, it was observed that there was a quantitative correlation in antithrombic activity at 4 minutes' incubation in dilutions greater than one part in forty-five. There was little or no difference in the antithrombin of serum and plasma. In the ultimate test the incubated (56°C, 10 minutes) serum or plasma was diluted (usual dilutions, 1 part in 40, 50, or 60) to such an extent that 1 cc would inactivate 0.48 to 0.59 units of thrombin in 4 minutes at 28°C. In such dilutions no correction was necessary for the thrombin-neutralizing activity of oxalates. However, when the results of such a test were compared with the ability of undiluted serum or plasma to neutralize or inactivate thrombin the calculated result had to be multiplied by a correction factor of 5.28. The addition of small amounts of thrombin to undiluted serum or plasma was no index of the antithrombic activity because of the unexplained phenomenon that small fractions of a unit of thrombin may remain active for considerable time. One unit of antithrombin is defined as that amount which will neutralize or inactivate 1 unit of thrombin in 4 minutes incubated at 28°C.

TABLE I.
Normal Levels of Antithrombin in Human Subjects and Various Animals.

Source of blood	No. of subjects	Avg antithrombin units per cc	Range of variation units per cc*
Human, normal	34	90	74-115
Dog	15	87	74-105
Cat	5	96	79-117
Cow	11	95	82-119
Pig	4	103	101-109
Guinea pig	10	108	97-120
Rabbit	18	108	98-133
Rat (albino)	28	123	102-147

*Lowest and highest determinations.

The antithrombin of the serum and plasma of various species was then determined (Table I). Human serum and plasma contain between 74 and 115 units per cubic centimeter. In human subjects with the plasma prothrombin within the hemorrhagic zone the prothrombic unitage usually approximated or was lower than the antithrombic unitage.

Summary. A method is devised for the quantitative determination of antithrombic activity of serum and plasma. One unit of antithrombin is defined as that amount which will inactivate or neutralize 1 unit of thrombin in 4 minutes' incubation at 28°C. There is little or no quantitative difference in the antithrombin of serum and plasma. The antithrombic activity of normal serum and plasma is considerably greater than has been previously described.

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Diphtheria-Antitoxin Production After Intravenous or Subcutaneous Injection of Alum-Toxoid.

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The purpose of the present study is to compare the effectiveness of intravenous and subcutaneous injections of alum-precipitated diphtheric toxoid in stimulating antitoxin-formation in the rabbit. The general opinion that the subcutaneous is superior to the intravenous route in antitoxin-production is based on experiments with horses[1] and guinea pigs.[2] Further important difference between the previous and the present experiments is that in the previous studies the antigen, toxin or toxoid, was used in solution and in the present study the toxoid was employed as particulate material.

When bacterial suspensions are employed as antigen the vascular route is the more effective one. This is true not only in regard to pneumococci that rapidly become gram-negative when introduced into the skin,[3] but also in regard to heat-killed tubercle bacilli that

1 Kolle, W., and Wassermann, A., *Handbuch der path. Mikroörganismen*, 1912, Verlag Gustav Fischer, Jena.

2 Neill, J. M., Sugg, J. Y., and Richardson, L. V., *J. Immunol.*, 1935, **28**, 363.

3 Julianelle, L. A., *J. Exp. Med.*, 1930, **51**, 441, 449; Dubos, R. J., and MacLeod, C. M., *J. Exp. Med.*, 1938, **67**, 269.