quantitative analysis. O'Brien and Peters[3] showed that oxygen consumption is moderately reduced in the avitaminous brain coupled with impairment of carbohydrate metabolism. This parallelism to insulin shock is further shown by the changes in brain potentials recorded here and, under insulin, by Dubner and Gerard,[4] Himwich *et al.*,[5] and others.

10778 P

Further Experience With the Use of Thrombin as a Hemostatic Agent.*

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We have recently reported animal experiments[1] on the use of purified thrombin[2] as a hemostatic agent. We are now able to obtain thrombin solutions free of bacteria, and with minor loss of activity, by use of fritted glass filters.[3] We wish to report additional toxicity experiments; also preliminary experience with the use of thrombin in a small group of human cases.

Toxicity Experiments. As pointed out previously,[1] thrombin, applied to operative surfaces, is non-toxic. Numerous other toxicity experiments with thrombin have since been performed, and a small group of these is presented below.

(1) Intramuscular administration. An injection of 6000 units into the thigh muscles of an adult rat produced no local thrombosis, and no clinical evidence of toxicity. Blood samples drawn several

3 O'Brien, J. R., and Peters, R. A., *J. Physiol.*, 1935, **85**, 454.

4 Dubner, H., and Gerard, R. W., *J. Neurophysiol.*, 1939, **2**, 142.

5 Himwich, H. E., Hadidian, Z., Fazekas, J. F., and Hoaglund, H., *Am. J. Physiol.*, 1939, **125**, 578.

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1 Seegers, W. H., Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Science*, 1939, **89**, 86.

2 Seegers, W. H., Brinkhous, K. M., Smith, H. P., and Warner, E. D., *J. Biol. Chem.*, 1938, **126**, 91.

3 Morton, Harry E., and Czarnetzky, E. J., *J. Bact.*, 1937, **34**, 461.

hours later showed no alteration in the plasma fibrinogen or prothrombin levels.

(2) Intraperitoneal administration. An injection of 6000 units of thrombin (4 cc) caused some uneasiness in a 300 g rat, possibly because the solution was hypotonic. The blood, 6 hours later, showed almost complete disappearance of the fibrinogen, and a 50% reduction in the plasma prothrombin. Intraperitoneal injection of 1500 units into a 200 g rat produced a very slight reduction in the plasma fibrinogen; 700 units had no effect. A proportionate dose in man would be at least 100 cc of concentrated thrombin solution.

(3) Intravenous administration. Rapid injection of 0.5 cc thrombin (250 units) into the jugular vein of a 300 g rat resulted in death in 60 seconds. Thrombi were found in the right ventricle and in the pulmonary artery. Injection of 100 units into a 285 g rat produced no obvious disturbance.

Thrombin, intravenously, is highly dangerous, yet the rat will tolerate larger amounts than one might expect, for 100 units of thrombin will clot 50 cc of oxalated blood *in vitro* in 16 seconds.

Hemostasis in Man. Because of difficulties in preparing large amounts of thrombin on a laboratory scale, the accumulation of experience in human cases is necessarily slow. We have now used thrombin in 21 human cases, 4 of which are tabulated to show its hemostatic possibilities.

Effective hemostasis depends upon formation of clots which seal the ends of tiny vessels. Large vessels must be ligated. If oozing is slow, prompt hemostasis can usually be obtained by applying thrombin with an atomizer. If the bleeding is brisk, however, the clot which forms often fails to adhere to the tissue, thus permitting

TABLE I.

Case No.	Bleeding Site	Thrombin units/cc	Method of application	Results
1	Operative, radical mastoidectomy	1500	Spray atomizer	Filmy clots formed in few seconds. Bleeding controlled with 1-3 applications.
2	Skin graft, donor areas, excessive oozing	1500	Spray atomizer	Hemorrhage effectively checked. Some areas required second application.
3	Two tooth sockets, bleeding 24 hr (hemophilia)	2500	Jet from hypodermic needle	Hemorrhage promptly checked.
4	Tiny stab wound, ear ("pseudohemophilia")	1000	Dropped into wound	Hemorrhage promptly checked.

continued oozing underneath the clot. In fact, the clot on the surface acts as a barrier between the fluid blood and the thrombin being applied. In such instances more satisfactory results are often obtained by applying the thrombin in a fine jet under pressure, thus forcing the thrombin into contact with the tissues. Further studies with this and other methods of application are being made.

10779

Inactivation of Prothrombin by Purified Thrombin Solutions.*

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In some recent experiments on the conversion of prothrombin into thrombin, we discovered a new reaction in which prothrombin is inactivated. It is our present purpose to present data regarding this inactivation, and to discuss the probable nature of the reaction.

Methods. Thromboplastin: Mix 100 g fresh ground beef lung with 100 cc saline. Allow to stand, with occasional stirring, for 48 hours at 5°. Centrifugalize and dilute the fluid obtained with an equal volume of saline. Any prothrombin present is removed by adsorption (one-sixth volume of $Mg(OH)_2$ suspension[1]) followed by centrifugalization. To 100 cc of the clear adsorbed solution add 100 cc $(NH_4)_2SO_4$ solution (saturated at 5°). Centrifugalize and dissolve the precipitate, containing thromboplastin, in 100 cc saline. Repeat the precipitation, and dissolve the final precipitate in 15 cc saline. Dialyze against saline until free of $(NH_4)_2SO_4$. The entire procedure is carried out in the cold room (5°). The product used in the present series of experiments contained 26 mg organic solids per cc.

To prepare an isotonic buffer solution (pH 7.25) which does not interfere with the action of calcium ion, dissolve 1.72 g imidazole (Eastman Kodak) in 90 cc of 0.1 N HCl, and dilute to 100 cc with H_2O.

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[1] Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.