An additional 30 minutes were allowed to elapse, and a lethal dose (LD_{99}) of the HPT proved immediately fatal to 8 of the 10 mice, with delayed death in the remaining 2. This indicates that prothrombin was still present, and substantiates the exhaustion of fibrinogen as the cause of the refractory state. The fact that the desensitization is lost gradually over a period of 8 to 12 hours is consistent with the known rate of regeneration of fibrinogen by the liver.

Conclusions. 1. Intravenous sublethal doses of human placental thromboplastin produce a refractory state in mice. During this state, the animals tolerate lethal doses of thrombin as well as thromboplastin, and the blood is incoagulable.

2. The refractory state is due to an exhaustion of the circulating fibrinogen. It may be abolished by restoring fibrinogen to the circulating blood, indicating that prothrombin is still present.

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Value of Soybean Trypsin Inhibitor in Preventing the Toxic Effects of Human Placental Thromboplastin.*

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As early as 1914, Gaifami[1] demonstrated that saline extracts of the human placenta, injected intravenously, are lethal to animals. Obata[2] investigated these effects extensively and showed, furthermore, that human serum neutralizes the toxic effects when mixed with the extract *in vitro*. Such lethal activity is not limited to placental tissue, for Krichesky and Mahler[3] observed toxic effects from extracts of rabbit uteri, but only in the progestational or gravid state.

Gizelt[4] showed the presence of thromboplastin in the human placenta, and Chargaff[5] has indicated by his recent quantitative studies that the thromboplastin content of the placenta is as high or higher than that of lung or brain. Schneider, in a series of studies on the lethal effect of tissue extracts, finally concluded[6] that the toxicity of placental extracts is entirely attributable to the thromboplastin content. He demonstrated that heparinization protects mice against such effects, and that the addition of heparin to the extracts *in vitro* likewise neutralizes the "placental toxin."

These facts have certain clinical implications pertaining to the origin of at least a part of the eclamptic syndrome in women. The circulatory arrangement of the human placenta is such that the maternal blood comes directly in contact with the trophoblastic epithelium, and the sequence of events which might arise as the result of a relative ischemia of the gravid uterus has been recently reviewed.[7] In eclampsia, the liberation of placental thromboplastin into the maternal circulation might well account for the deposition of fibrin observed especially in the liver. Preliminary reports, furthermore, indicate that heparinization may be of value in the treatment of preeclampsia.[8,9] There are, on the other hand, several disadvantages to

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1 Gaifami, P., *Z. Biochem. u. Biophys.*, 1914, **17**, 74.

2 Obata, I., *J. Immunol.*, 1919, **4**, 111.

3 Krichesky, B., and Mahler, J., *Endocrinology*, 1942, **30**, 616.

4 Gizelt, A., *Arch. ges. Physiol.*, 1913, **152**, 562.

5 Chargaff, E., *J. Biol. Chem.*, 1945, **161**, 389.

6 Schneider, C., *Am. J. Physiol.*, 1947, **149**, 123.

7 Page, E. W., *Obs. and Gyn. Survey*, in press.

TABLE I.
Protective Action of Soybean Trypsin Inhibitor.

STI (0.5 g/kg) administered	No. of mice	Mg (dry wt) of HPT (lethal dose)	Time (min.) between STI and HPT	Toxic effects: Immediate No.	Immediate %	Delayed No.	Delayed %	None No.	None %
None (controls)	28	0.8	—	28	100	0	0	0	0
Intravenously	24	0.8	30-45	2	8.3	1	4.2	21	88
Intramuscular	18	0.8	120-150	2	11	3	16.6	13	72
Subcutaneously	12	0.8	180+	6	50	6	50	0	0
I.V. (after mixing with HPT)	10	0.8	—	0	0	1	10	9	90

STI = Soybean Trypsin Inhibitor. HPT = Human Placental Thromboplastin. The differences between the immediate and delayed toxic effects of the HPT are explained in the text.

the clinical employment of heparin over long periods of time.

Macfarlane,[10] utilizing a crystalline soybean trypsin inhibtor prepared by Kunitz,[11] showed that this protein inhibited the coagulation of blood by a "depression of the activity of thromboplastin." The purpose of the present study is to determine whether the soybean trypsin inhibitor would protect against the lethal effects of human placental extracts.

Materials and methods. The human placental thromboplastin (HPT) solution used† is the same as that described in the previous communication.[12] The dry weight of the dialyzed material (protein content) was 18.5 mg/ml.

The soybean trypsin inhibitor‡ (STI) is approximately 50% as pure as the crystalline material, and when assayed against fibrinolysin by the method of Loomis, *et al.*,[13] contained 150 units/mg. A 6% solution was prepared in sterile 0.9% saline.

Mice of either sex, weighing between 20 and 30 g, were used for the toxicity studies. All injections of the HPT were given intravenously in volumes not exceeding 0.3 ml. Estimations of the lethal dose (LD_{99}) were made as in the previous report.[12] The STI solutions were administered to mice by several routes, as indicated in Table I, and in addition were given intravenously to a guinea pig, rabbit and monkey. The *in vitro* tests were made by incubating the HPT and STI solutions together at 37° C for 30 minutes prior to injection.

Results A. *Soybean trypsin inhibitor alone is not toxic.* An amount of STI equivalent to 0.5 g/kg body weight was given intravenously to 30 unanesthetized mice without any observable evidence of immediate or delayed toxicity. To evaluate the sensitivity of other species, 0.5 g per kilo were given intravenously to a guinea pig and to a rabbit and 0.25 g/kg to a Rhesus monkey. There was no observable reaction, and when each of these 3 animals were reinjected with a smaller dose 2 weeks later, no anaphylactic response was noted. In the case of the monkey, blood samples drawn with silicone coated syringes and studied by the method of Jaques *et al.*[14] showed a 4-fold increase in the plasma coagulation time a half hour after the initial injection of the STI.

B. *Soybean trypsin inhibitor protects mice against the toxic effects of placental thromboplastin.* The stock HPT solution was diluted with 3 volumes of saline in order to increase the accuracy of measurement.

[8] Maeck, J., and Zilliacus, H., *Am. J. Obstet. and Gynecol.*, 1948, **55**, 326.

[9] Page, E. W., *Am. J. Med.*, 1948, **4**, 784.

[10] Macfarlane, R., *J. Physiol.*, 1947, **106**, 104.

[11] Kunitz, M., *J. Gen. Physiol.*, 1947, **30**, 291 and 311.

† We are indebted to Dr. F. F. Johnson of Cutter Laboratories, Berkeley, California, for supplying the placental thromboplastin.

[12] Fulton, L., and Page, E. W., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 594.

‡ The soybean trypsin inhibitor was prepared and given to us by E. C. Loomis, Research Laboratories, Parke, Davis & Co., Detroit.

[13] Loomis, E. C., George, C., Jr., and Ryder, A., *Arch. Biochem.*, 1947, **12**, 1.

[14] Jaques, L., Fidlar, E., Fecsted, E., and MacDonald, A., *Can. Med. Assn. J.*, 1946, **55**, 26.

Estimations of the lethal dose, based on extrapolated values obtained with logarithmic-probit paper, indicated that 0.3 ml (equivalent to 0.8 mg dry weight of protein) would theoretically kill 99% of animals. All 28 mice receiving this dose died within 3 minutes. The STI solutions were given intravenously to 24, intramuscularly to 18 and subcutaneously to 10 animals. The results are given in Table I. Two endpoints were observed; (1) immediate death, and (2) a delayed reaction consisting of convulsions, or dyspnea or death within the subsequent 24-hour period. The intravenous administration of the soybean material appeared to offer the greatest degree of protection, since 88% of the group showed no reactions of either type to lethal doses of HPT. The intramuscular route offered a moderate protection providing that two hours elapsed before giving the HPT. The subcutaneous route was far less effective. When the STI and HPT solutions were incubated *in vitro* before injection, there was almost complete neutralization of the toxic effects.

In an attempt to determine the effective dosage of STI, the amount was reduced by half (*i.e.* from 18 mg to 9 mg) and given intravenously to an additional 12 mice. Two of the animals died within 5 minutes, and 9 additional mice showed delayed reactions. The lower dose level, therefore gives incomplete protection.

Conclusions. 1. When given intravenously to 4 species, the soybean trypsin inhibitor, in doses up to 0.5 g/kg, was devoid of any apparent toxic or anaphylactic effect. In the monkey, it was noted that the material caused a prolongation of the plasma coagulation time.

2. Given either intravenously or intramuscularly, the soybean trypsin inhibitor offered considerable protection against the subsequent administration of lethal doses of human placental thromboplastin, as shown in Table I.

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Inhibition of Cholinesterase by Adrenaline.

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Considerable evidence has accumulated which indicates that the neuro-humoral agents, acetylcholine and adrenaline, are not entirely antagonistic substances. Although the effector response to these two substances in most visceral structures is physiologically opposite, observations have suggested that in the central nervous system, in autonomic ganglia and at somatic motor nerve endings the one substance, adrenaline, may modify the action of the other and behave as a synergist.

Numerous experiments which have been reviewed by Burn[1] demonstrate the occurrence of this phenomenon. Dale and Gaddum,[2] in working with denervated skeletal muscle, demonstrated that the effect of acetylcholine in causing contracture could be augmented by prior administration of adrenaline. von Euler and Gaddum[3] and Bulbring and Burn[4] have similarly demonstrated increased contracture in skeletal muscle by adrenaline.

In attempting to demonstrate that acetylcholine is responsible for the transmission of impulses in the spinal cord, Bulbring and

[1] Burn, J. H., *Physiol. Rev.*, 1945, **25**, 377.

[2] Dale, H. H., and Gaddum, J. H., *J. Physiol.*, 1930, **70**, 109.

[3] v. Euler, U. S., and Gaddum, J. H., *J. Physiol.*, 1931, **73**, 54.

[4] Bulbring, E., and Burn, J. H., *J. Physiol.*, 1936, **86**, 61.

[5] Bulbring, E., and Burn, J. H., *J. Physiol.*, 1941, **100**, 337.