TABLE I.
Poliomyelitis Virus in Stools Before Onset of Symptoms.

Name	CSA*	Date of onset and symptoms at onset	Dates of collection of stools tested	Virus isolations
El Bal	WF2½	8/5/46 Fever of 99.6° Axillary 1 day only	8/4/46 and 8/5/46 7/30/46 and 7/31/46 7/27/46	Virus isolated Virus not isolated Virus isolated 9 days before onset
Jo Jac	WF3	8/15/46 Fever 99.6° Axillary 1 day only	8/14/46 and 8/21/46 8/8/46 7/27/46	Virus isolated Virus isolated 7 days before onset Virus isolated 19 days before onset
Ri Sic	WM3½	8/7/46† Drowsiness and constipation	8/1/46, 8/5/46, and 8/8/46 7/26/46	Virus isolated Virus isolated 12 days before onset
Pa Woz	WF4	7/29/46 Fever	7/29/46 and 8/1/46 7/27/46	Virus isolated Virus not isolated

* Color, Sex, Age.

† This child had no stool on 7/27/46. Previous analysis of symptoms has indicated that constipation in contacts is of significance only if it occurs on successive days or, if on one day only, in conjunction with some other symptom typical of poliomyelitis (*i.e.*, fever, headache, myalgia, vomiting, or drowsiness).

stool of one child 19 days and 7 days before the onset of the clinical disease; from the stool of a second child 9 days before onset of the clinical disease, and from the stool of a third child 12 days before the onset of the clinical disease.

The onset dates (8/5, 8/7, and 8/15) in the 3 children with virus in stool prior to appearance of symptoms were the latest recorded among the 22 children in the nursery school who had the clinical disease. This probably indicates prolonged incubation periods in these 3 children, since no symptoms or temperature rises were detected by earlier daily observations in the school beginning on July 25. The other children, including the one paralytic and the 2 meningitic cases, had onsets between July 14 and August 5.

These observations confirm those reported by other workers, namely, that the virus of poliomyelitis may be present in the stools of man for a considerable period of time prior to the onset of symptoms of the disease.

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Nature of the Refractory State Following Sublethal Dose of Human Placental Thromboplastin.*

LEE D. FULTON AND ERNEST W. PAGE.

From the Division of Obstetrics and Gynecology, University of California Medical School, San Francisco.

The toxicity of placental extracts upon intravenous injection into experimental animals has been studied repeatedly in the past half century by investigators interested in the toxemias of late pregnancy. Schneider,[1] however, was the first to report on the refractory state in mice following sublethal injections of "placental toxin." This desensitization was temporary and lasted for ap-

* Aided by grants from the John and Mary Markle Foundation, New York City, and the James Fund of the University of California Medical School.

1 Schneider, C., *Am. J. Physiol.*, 1946, **147**, 250.

proximately 8 to 12 hours. Animals became refractory almost immediately after intravenous injection of sublethal doses, and were then able to withstand 20 times the minimum lethal dose. Following the second injection, about 95% of the mice were found to have liver lesions consisting of widespread focal necrosis.[2] No explanation for the refractory state was offered other than that it "involved a change in some portion of the reacting mechanism itself."

Subsequently, Schneider[3] identified the toxic principle of placental extracts as thromboplastin. This would suggest that the refractory state might be due to the exhaustion of one or more of the essential components of the coagulation system.

Materials and methods. Crude placental thromboplastin† was prepared by extracting a human placenta with cold 0.9% sodium chloride solution in a Waring blender at pH of 7.1. The mass was strained through cheese cloth, and the insoluble material re-extracted in the same manner. The combined extracts were diluted with 5 volumes of cold water, and brought to a pH of 5.1 with 0.1 N. HCl. The precipitate, removed by centrifugation, was resuspended in 100 ml of distilled water, made isotonic with sodium chloride and adjusted to a pH of 7.4. The dry weight of the nondialyzable solids (protein) was 18.5 mg/ml of solution.

Commercial bovine thrombin (Upjohn) was dissolved in sterile saline to a strength of 200 units/ml. Bovine fibrinogen (Plasma Fraction I, Armour, 75% clottable) was prepared as a 2% solution in physiological saline.

Mice of either sex, weighing between 20 and 30 g, were used as test animals. All injections were given intravenously into the caudal veins, utilizing the technic of Nickson and Barkulis.[4] Sublethal doses (LD_{50}) of human placental thromboplastin (HPT) were calculated by interpolation on logarithmic-probit paper[5] utilizing 6 animals at each dose level. Only those doses which killed less than 90 or more than 10% of each group were used in the calculation. From the best fitting line, the LD_{50} was determined as well as the LD_{99}, *i.e.*, that dose which would theoretically kill 99% of an infinitely large sample. Actually, this latter dose killed all control animals.

Results. A. *Incoagulability of the blood during the refractory state.* Following the injection of the sublethal dose (LD_{50}) 30 surviving mice were given the lethal dose and all survived, thus substantiating the existence of the refractory state. Nine of these animals showed liver lesions, similar to those described by Schneider, providing they were sacrificed within 48 hours. In an additional group, killed after one week, no lesions were observed. During the refractory state the blood would not clot *in vitro* even after the addition of thrombin or thromboplastin. This suggested that the desensitization was due to the exhaustion of fibrinogen.

B. *Resistance to fatal doses of thrombin in the refractory state.* Twelve animals, surviving the LD_{50} dose of HPT were injected an hour later with a dose (50-60 units) of thrombin, an amount fatal to all control animals. None of this group showed any immediate observable effects, although one animal expired 12 hours later. While this experiment substantiated the fact that the fibrinogen was exhausted, it did not establish whether the prothrombin of the blood was likewise depleted.

C. *Abolition of the refractory state by the restoration of fibrinogen.* In order to test the thesis that the refractory state was due only to fibrinogen loss unaccompanied by prothrombin depletion, the refractory state was induced in 10 mice as before, and one hour later 6 mg of fibrinogen were injected intravenously. (This dose was calculated to restore the approximate normal fibrinogen content of the blood in a 20 g mouse.)

[2] Schneider, C., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 325.

[3] Schneider, C., *Am. J. Physiol.*, 1947, **149**, 123.

† Human placental thromboplastin was generously supplied by Dr. F. F. Johnson of Cutter Laboratories, Berkeley, California.

[4] Nickson, J., and Barkulis, S., *Science*, 1948, **107**, 229.

[5] Miller, L., and Tainter, M., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 261.

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.*

LEE D. FULTON AND ERNEST W. PAGE.

From the Division of Obstetrics and Gynecology, University of California Medical School, San Francisco.

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

* Aided by grants from the John and Mary Markle Foundation, New York City, and the James Fund of the University of California Medical School.

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.