Effect of Soybean Phosphatide on Blood Coagulation Defect Following Total Body X-Irradiation in the Dog.* (20366)

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It has been reported that the active principle in platelets which contributes to the formation of thromboplastin from plasma sources resides in a phospholipid fraction of the platelets(1,2). The present investigation was conducted in order to determine whether soybean phosphatide can be used as a substitute for the platelet factor in dogs made thrombocytopenic by total body x-irradiation. Soybean phosphatide was chosen for this purpose because it is readily available and has been used previously to stabilize neutral fat emulsions and fat soluble vitamin K preparations for intravenous use in man(3).

Methods. Blood was obtained from the foreleg vein of the dog with one No. 18 gauge needle and 2 syringes coated with silicone oil.‡ Four and one-half ml of blood from the first syringe was added to 0.5 ml of 0.1 M potassium oxalate and used for plasma tests. The clotting time and prothrombin utilization tests were done on blood from the second syringe. *Whole blood clotting time (C.T.).* Two ml aliquots of blood were placed in each of two 13 by 100 mm glass tubes in a waterbath at 37°C. Starting at 3 minutes, both tubes were tipped every minute until each could be inverted without spilling the contents. The results were averaged. Incubation of the specimens at 37°C was continued until one hour had elapsed from the time the blood was drawn, at which time 0.22 ml of 0.1 M potassium oxalate was added to each tube and mixed with the clotted or clotting blood. The tubes were then promptly centrifuged to recover the serum for prothrombin testing. Clot retraction was observed at the end of one hour's incubation of the blood at 37°C. *Prothrombin assays on plasma and serum* were done by the 2-stage method of Ware and Seegers(4), using a lyophilized incubation mixture§ and bovine fibrinogen‖ in the test. A 1.25% solution of fibrinogen in 0.9% NaCl was adsorbed with freshly precipitated and washed barium sulfate in order to remove any traces of prothrombin. *Residual serum prothrombin determinations.* The number of units of prothrombin found in the serum was divided by the number of units of prothrombin found in the plasma and multiplied by 100 in order to determine the per cent residual serum prothrombin. A value of greater than 30% residual serum prothrombin is significant evidence of impaired prothrombin utilization. This test provides a sensitive index of the severity of the coagulation defect which occurs following total body x-irradiation(5-7). *In vitro assay of clotting properties of soybean phosphatide.* Two methods were employed: 1) 1.0 ml of blood was added to 0.1 ml of soybean phosphatide solution, and the effect on prothrombin utilization was determined. The results were compared with those produced by the addition of the blood to 0.1 ml of standardized tissue thromboplastin preparation.¶ 2) 0.1 ml of 0.025 M $CaCl_2$ was added to a mixture of 0.1 ml of dog plasma and 0.1 ml of soybean phosphatide solution in a 13 by 100 mm pyrex tube in a waterbath at 37°C. The clotting time of duplicate specimens was determined and the results averaged. The results were compared with those obtained when 0.1 ml of a standardized tissue thromboplastin preparation or 0.1 ml of 0.9% NaCl

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‡ G. E. silicone oil No. 9996-300.

§ Difco Laboratories, Inc., Detroit, Mich.

‖ Armour Laboratories, Chicago, Ill.

¶ Simplastin—Chilcott Laboratories, Morris Plains, N. J.

was substituted for the phosphatide solution in the test. *Platelet counts* were done by the method of Brecher and Cronkite(8). *Preparation of soybean phosphatide.* 400 g of soybean phosphatide** were dissolved in 1.6 liters of redistilled petroleum ether (B.P. 65°C) and filtered through an asbestos type bacterial filter. The filtrate was poured with rapid stirring into 4 liters of redistilled acetone and the precipitate collected on a filter paper. It was redissolved in petroleum ether and reprecipitated as before. The precipitate was washed well with acetone and freed of solvent by means of a stream of dry nitrogen. The product was a light tan-colored granular material, containing approximately 31% lecithin and 31% cephalin. The remainder was composed mainly of inositol phosphatides. For use in this study, the material was dissolved in 5% glucose in water to a concentration of 2.5%. *Technic of x-irradiation.*†† All dogs were given a total dose of 150 r of total body x-irradiation. This dose is LD_{50} in our laboratory. The irradiation was given in 2 doses of 75 r delivered to each side of the body, while the dogs were under light pentobarbital anesthesia. Radiation factors‡‡ were 250 KV constant potential, 12 ma, HVL 1.5 mm Cu; filter, 0.5 mm Cu and 1 mm Al. Target to skin distance (tsd) was 125 cm for all dogs. The mean mid-body dose rate of 14 r per minute was calculated for each dog individually by averaging equal numbers of Victoreen ionization-chamber readings taken on the proximal and distil skin surfaces at representative areas.

Results. 1) *In vitro effects on prothrombin utilization.* The effect on prothrombin utilization of the addition of soybean phosphatide and of tissue thromboplastin to the blood *in vitro* is shown in Fig. 1. Blood obtained from a male dog, weighing 13 kg, 21 days after 150 r total body x-irradiation, in which no platelets were seen, was used in this experiment. The

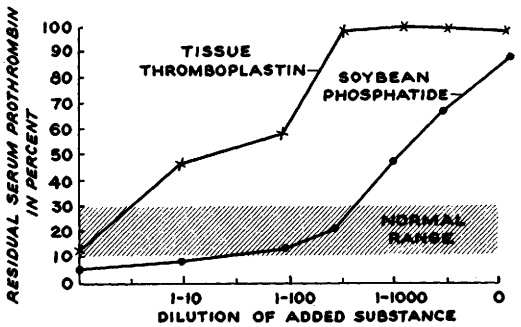

FIG. 1. Effect of addition of soybean phosphatide and of tissue thromboplastin on prothrombin utilization in the thrombocytopenic x-irradiated dog. (Serum tested after incubation one hr at 37°C of 1 ml of blood mixed with 0.1 ml of each dilution of substance tested. Prothrombin determinations by 2-stage method of Ware and Seegers(4).)

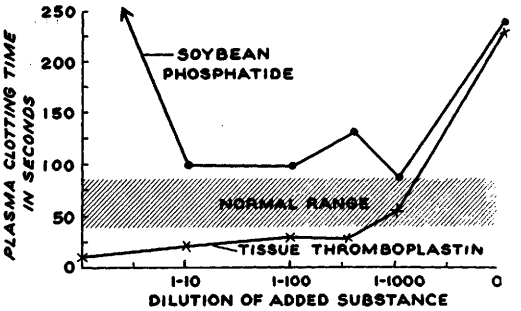

FIG. 2. Effect of addition of soybean phosphatide and of tissue thromboplastin on plasma clotting time of the thrombocytopenic x-irradiated dog. (One ml 0.025 M $CaCl_2$ added to 0.1 ml of plasma and 0.1 ml of substance tested at 37°C.)

soybean phosphatide solution produced a more profound effect, which was less rapidly lost by dilution, than did the tissue thromboplastin. Neither substance induced clot retraction.

2) *In vitro effects on plasma clotting time.* The effect on the plasma clotting time of the addition of soybean phosphatide and of tissue thromboplastin to the plasma of the same dog is shown in Fig. 2. The soybean phosphatide solution produced less shortening of the clotting time than did the tissue thromboplastin. In concentrated solution it proved to have anticoagulant properties. In this type of test the clot-promoting properties are apparently obscured by a clotting inhibitor in the phosphatide solution.

3) *Effect of slow intravenous infusion.* A male dog, weighing 13 kg, under light pentobarbital anesthesia, 8 days after 150 r total

** Grade "RG" soybean lecithin, Glidden Co., Chicago, Ill.

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‡‡ Westinghouse Quadroncondex Machine.

TABLE I. Effect of Intravenous Infusion during One Hour of 37 ml of 2.5% Soybean Phosphatide Solution (Diluted to 150 ml with 5% Glucose) on Blood Coagulation Defect in the Thrombocytopenic X-Irradiated Dog. No clot retraction in each experiment.

	Clotting time, min.	Platelet count/mm³ × 1000	Plasma prothrombin in units/ml*	Residual serum prothrombin in units/ml*	Residual serum prothrombin in % of plasma value
Before infusion	17	37	170	86	50
15 min. after infusion started	5		200	27	13
30 min. after infusion started	5	28.5	130	26	20
60 min. after infusion started (end of infusion)	<3		105	<10	<10
2 hr after end of infusion	7	38	140	23	16
22 hr after end of infusion	16	11	120	72	59

* Two-stage method of Ware and Seegers (4).

TABLE II. Effect of Intravenous Infusion during a Period of 25 Minutes of 100 ml of 2.5% Soybean Phosphatide Solution on Blood Coagulation Defect in the Thrombocytopenic X-Irradiated Dog. No clot retraction.

	Clotting time, min.	Platelet count/mm³ × 1000	Plasma prothrombin in units/ml*	Residual serum prothrombin in units/ml*	Residual serum prothrombin in % of plasma value
Before infusion	19	11.5	155	92	58
15 min.†	8	12	72	18	25
1½ hr†	14	10.5	90	45	50
3½ hr†	18		73	92	126

* Two-stage method of Ware and Seegers (4).
† After end of infusion.

body x-irradiation, whose blood showed 37000 platelets/mm³, no clot retraction, and a 17-minute C.T., was given 37 ml of the phosphatide solution diluted to 150 ml with 5% glucose in water by intravenous infusion in a period of 60 minutes. The results are shown in Table I. At the termination of the infusion, the C.T. was reduced to less than 3 minutes, and the residual serum prothrombin to less than 10%. Marked effects persisted for 2 hours, but had disappeared by 22 hours. A non-anesthetized female dog, weighing 11.5 kg, 11 days after 150 r total body x-irradiation, whose blood showed 11,500 platelets/mm³, no clot retraction, and a 19-minute C.T., was given 100 ml of the 2.5% phosphatide solution intravenously over a period of 25 minutes. The results are shown in Table II. Fifteen minutes after the infusion was completed, the C.T. was reduced to 8 minutes and the residual serum prothrombin to 25%. Both values had returned to the pre-infusion level at the end of 3½ hours. The platelet count and clot retraction remained unchanged. In both of these experiments the plasma prothrombin was also reduced by the infusion of the soybean phosphatide solution.

4) *Effect of small rapid intravenous infusion.* A male dog, weighing 13 kg, 9 days after 150 r total body x-irradiation, whose blood showed 11,500 platelets/mm³, no clot retraction, and a 14-minute clotting time, was given 20 ml of the 2.5% soybean phosphatide solution intravenously in a period of 3 minutes. The C.T. was reduced to 8 minutes during the observation period. There was no change in the platelet count or clot retraction. The effect on the residual serum

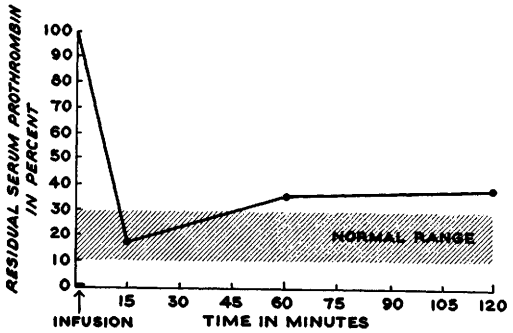

FIG. 3. Effect of rapid intravenous infusion of 20 ml of a 2.5% soybean phosphatide solution on prothrombin utilization in the thrombocytopenic x-irradiated dog. (Serum tested after incubation of blood for one hr at 37°C. Prothrombin determinations by 2-stage method of Ware and Seegers(4).)

prothrombin is shown in Fig. 3.

Summary. Thrombocytopenia, prolonged whole blood clotting time, and markedly impaired clot retraction and prothrombin utilization were induced in dogs by 150 r total body x-irradiation. A marked increase in prothrombin utilization was found after the addition of a soybean phosphatide solution to the blood *in vitro*. A temporary decrease in the clotting time and an increase in prothrombin utilization, without change in the platelet count or clot retraction, was induced by the intravenous administration of 20 to 100 ml of a 2.5% solution of the soybean phosphatide.

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Effect of Glucagon in Stable and Unstable Diabetic Patients. (20367)

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A hyperglycemic factor, termed "glucagon" by Murlin *et al.*(1), has been known for 30 years to be present in most commercial insulin preparations. Its isolation, purification, and characterization has been an exceedingly stubborn problem. Recently, the authors have had available for study a preparation of an unusually high degree of purity provided by A. Staub of these Laboratories(2). The material used in this study was of such potency that 0.5 μg of the substance per kg administered intravenously to unfed cats anesthetized with amobarbital produced a rise in the blood sugar of 30 mg per 100 cc above normal within 25 minutes (a "cat unit"). Further animal assays showed it to contain only 0.005 unit of insulin per mg of solid.

During the course of an investigation of the physiological properties of this highly purified hyperglycemic-glycogenolytic factor (glucagon), the authors studied the response of various types of diabetic subjects. In this report only the response of the blood sugar and serum inorganic phosphorus will be discussed. Data relative to pyruvate, lactate, potassium, and certain steroid studies will be presented elsewhere(3).

Technic. An intravenous infusion of diluted glucagon was given both to normal persons and to 8 female diabetic patients. All subjects received 10 μg/kg body weight (10 μg of this material was equivalent to 20 "cat units") in the fasting state; the diabetics had not received insulin on the morning of the test. Since no dose exceeded 1 mg of the test material, no one received more than 0.005 unit of insulin based on animal assay results. Venous blood sugar was determined by the