spiral antiserum and complement, and description of the HL procedure is presented. The HL procedure was shown to be broadly specific, in sharp contrast to the high degree of type specificity seen in the AL procedure. In comparison with HL titrations, the HA reaction was also found to have broad specificity but to be considerably less sensitive than the HL reaction. All 3 procedures were compared in a study involving 580 randomly selected human sera, from which data it would seem that the HL procedure warrants further study as a possible screening tool for the rapid detection and titration of leptospiral antibodies in human sera.

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Failure of Synthetic 5-Hydroxytryptamine Creatinine Sulfate to Enhance Clot Retraction of Platelet-Poor Human Plasma.* (22114)

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Many platelet constituents with specific effects on the hemostatic mechanism have been recently identified(1). Whether they represent intimate constituents of platelets or are carried by them still requires investigation. These agents include a thromboplastic factor (2), constituents accelerating conversion of prothrombin to thrombin and of fibrinogen to fibrin(3), an antiheparin factor(4), possibly antifibrinolysin(5,6). An additional substance, 5-hydroxytryptamine (serotonin), has been identified in dried platelets preparation (7,8) and shown to liberate from platelets during clotting of human blood(9). Serotonin has been shown to possess vasoconstrictor and capillary protecting activity(10). Recently, it has also been suggested that serotonin is able to induce clot retraction of platelet-poor bovine and human plasma(11,12). This observation is of considerable importance since clot retraction has been generally considered to be due to intact platelets rather than to a single platelet constituent. This study is an attempt to evaluate the clot retraction promoting effect of the commercially available synthetic derivative of serotonin, 5-hydroxy-tryptamine creatinine sulfate, the use of which is currently recommended in the treatment of the bleeding tendency of thrombocytopenic states. At the same time, observations have been made on the ability of this compound to influence the rate and extent of prothrombin conversion during clotting of platelet-poor plasma.

Materials. Native platelet-poor plasma.

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TABLE I. Effect of 5-Hydroxy-tryptamine Creatinine Sulfate on Clot Retraction and Prothrombin Utilization during Clotting of Undiluted Platelet-Poor Human Plasma.*

Final conc. of 5-hydroxy-tryptamine creatinine sulfate, mg %	Clot retraction, % (24 hr after clotting)	Prothrombin activity of serum, % (1 hr after clotting)
80	10.5	87
40	10	84
20	9.5	86
10	10	92
5	8	81
2.5	8.5	84
1.25	9	93
.6	9.5	93
.3	10	87
.1	10	90
Control†	8	90

* Avg results of 4 experiments.
† Saline solution, 1/10 volume.

Blood was collected from fasting healthy donors with the "double syringe technic" in Silicone-coated syringes through Arquad 2-C coated needles. Blood was transferred to pre-cooled Silicone-coated test tubes and centrifuged in the cold (4°C) at 3,000 for 30 minutes. The upper ⅔ of supernatant plasma only were then promptly transferred to another pre-cooled Silicone-coated test tube and promptly added to the various dilutions of the substance under study. Silicone-coated pipettes were used in all steps. *Citrated platelet-poor plasma.* Blood was collected with the same technic and transferred to Silicone-coated test tubes containing 1/10 volume of 0.2 M solution of sodium citrate. Blood and anticoagulant were promptly mixed by inverting the tube which had been stoppered with paraffin-coated cork. *Synthetic 5-hydroxy-tryptamine creatinine sulfate,* was made available through the courtesy of Dr. Merrill E. Speeter.§ Serial dilutions were made in saline solution, and strengths used are indicated in Tables I and II. *Determination of clot retraction.* Immediately after completion of clotting, clot was rimmed by the technic of pushing a thin glass rod to the bottom and circling it around the clot. Tubes were then incubated in water bath at 37°C for 1 or 3 hours. After visual inspection, volume of serum expressed when the tube was inverted at an angle of 60° was taken as measure of retraction. *Serum prothrombin time (prothrombin consumption)* was determined by our method(13), 1 or 3 hours after completion of clotting.

Results. 1. *Effect of 5-hydroxy-tryptamine creatinine sulfate on clot retraction and prothrombin utilization during clotting of platelet-poor native human plasma.* Experiments were made in duplicate. Various concentrations of 5-hydroxy-tryptamine creatinine sulfate in a constant volume of 0.1 ml were transferred to chemically clean glass test tubes kept in water bath at 37°C; 0.9 ml of native platelet-poor human plasma were then added to each tube and the contents mixed. A similar experiment was performed using Silicone-coated test tubes and comparable results were obtained. There was no appreciable and constant effect on the speed of clotting, clot retraction and serum prothrombin activity one and 3 hours respectively after completion of coagulation. There was no appreciable difference in clot retraction 24 hours after clotting between tubes containing 5-hydroxy-tryptamine creatinine sulfate and the control containing 1/10 volume of saline solution, as shown in Table I.

2. *Effect of 5-hydroxy-tryptamine creatinine sulfate on clot retraction of diluted native or citrated human plasma.* Native or citrated human plasma were diluted 1:5 with sterile 0.85% NaCl solution. 4.5 ml of diluted plasma were added to a series of glass test tubes kept in water bath at 37°C con-

TABLE II. Effect of 5-Hydroxy-tryptamine Creatinine Sulfate on Clot Retraction and Prothrombin Utilization during Clotting of Diluted Platelet-Poor Human Plasma.*

Final conc. of 5-hydroxy-tryptamine creatinine sulfate, mg %	Clot retraction, % (24 hr after clotting)	
	Citrated plasma†	Native plasma
40	74	
20	72	No clot
10	70	
Control‡	72	

* Avg of several experiments.
† Clotted with bovine thrombin.
‡ Containing saline solution, 1/10 volume.

§ Director of Biochemical Research, Upjohn Co.

taining various concentrations of 5-hydroxy-tryptamine creatinine sulfate in a constant volume of 0.5 ml. Diluted native plasma was allowed to clot spontaneously. Clotting often did not occur in some instances when plasma had been made practically platelet-free by centrifugation. Diluted citrated plasma was clotted with the addition of 0.2 ml saline solution containing 60 units of purified bovine thrombin. Clot retraction was observed and volume of separated serum measured 3 and 24 hours after completion of coagulation. Results 24 hours after clotting are presented in Table II. Residual prothrombin activity was negligible because of the high dilution of plasma. There was no demonstrable effect on retraction of the clot.

Discussion. Correll *et al.*(10) studied the thromboplastic activity of serotonin derivatives by investigating their ability to accelerate the clotting time of plasma. Their negative results are confirmed by ours. On the other hand, our results on effect of serotonin derivatives on the retraction of the clot are not confirmatory of those already presented in the literature(11,12). Since, however, it is not known whether synthetic products and 5-hydroxy-tryptamine carried or liberated by platelets are identical in structure and properties, our negative experiment does not prove that platelet serotonin may not contribute to the mechanism of clot retraction, as postulated by Fenichel and Seegers.

Perhaps, explanations might be offered for the discrepancy between our findings and those of other authors. There may be differences in purity of the reagents used. Moreover, the complexity and multiplicity of the factors influencing clot retraction has been emphasized by many authors and recently summarized by Budtz-Olsen(14). Thus, it is likely that, without extremely careful control of all experimental conditions, results may be at great variance and not constantly reproducible. Since little is known of the ideal conditions under which clot retracts *in vitro* and *in vivo,* the evaluation of the clot promoting activity of a drug remains a hazardous problem.

Summary. Synthetic 5-hydroxy-tryptamine creatinine sulfate failed to enhance clot retraction and prothrombin utilization during clotting of platelet-free human plasma. These results are at variance with recently reported findings.

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