Effects of 5-Hydroxytryptamine on Some Aspects of Hemorrhagic State in Radiation-Induced Thrombocytopenia.* (23802)

I. Djerassi, E. Klein, Sidney Farber, D. Palmer
Children's Cancer Research Fn., Division of Laboratories, Children's Medical Center, and Department of Pathology, Harvard Medical School, Boston

The presence of serotonin in platelets has been related to their vasoconstrictor activity (1). The connection of this activity with hemostasis was investigated in a number of clinical studies(2,3,4). Experiments under standardized conditions, however, were confined to intact animals(5.6.7). Since disturbances of hemostasis emphasize the effects on the hemostatic mechanism, the action of serotonin on the bleeding in thrombocytopenia was investigated.

The effects of serotonin on increased vascular fragility and traumatically-induced bleeding in irradiated, thrombocytopenic animals are reported.

Methods and materials. 5-hydroxytryptamine-creatinine sulfate (Antemovis†) was used throughout these studies and was administered intraperitoneally to mice and guinea pigs. A 250 kv source with 0.25 mm copper and a1 mm aluminum filter was used for whole body X-irradiation of mice and guinea pigs. A dose of 325 r was given to the guinea pigs representing an LD_{50-60}, while 750 r (LD_{90-100}) was given to adult white Swiss-Webster mice. Vascular fragility was determined on the skin of guinea pigs by the negative pressure method(8). The number of petechiae formed under a negative pressure of 120-150 mm applied for 30 seconds was recorded. The time of bleeding induced by tail snips (approximately 1 mm from the tip) in Swiss-Webster mice was determined. All experiments were carried out in the period of maximal thrombocytopenia (6th-7th day for the guinea pigs and 7th-8th day for the mice).

Results. A. *Vascular fragility in irradiated, thrombocytopenic guinea pigs.* Vascular resistance of guinea pigs rendered thrombocytopenic by X-irradiation, was increased by the use of relatively large amounts of serotonin (Table I). Intravenous administration of the doses indicated in Table I produced marked pulmonary distress and occasional, transient convulsions lasting 2-5 minutes. The intraperitoneal injection of high doses of serotonin led to milder pulmonary reactions. Vascular fragility was affected only by doses of 5-hydroxytryptamine which were high enough to also produce pulmonary reactions. The type of response produced with 0.5 mg serotonin given intravenously is illustrated in Fig. 1.

B. *Duration of traumatic bleeding in irradiated, thrombocytopenic mice.* In these experiments serotonin was administered intraperitoneally in order to avoid local, vasospastic effects on the tail veins of the animals. Convulsions or respiratory distress were not observed. High doses of serotonin reduced the duration of bleeding (Table II). In the lowest effective dose range (0.25 mg) there was considerable variation in response. Shortening of the bleeding time was also observed in a control group of normal mice (Table II).

Discussion. Previous studies(5,6,7) have shown that 5-hydroxytryptamine increases vascular resistance and shortens the bleeding time in normal animals. This is consistent with the effects observed in thrombocytopenic animals in the present studies. The effective doses, however, for both normal and thrombocytopenic animals were high. The rate of administration of serotonin was found to be an important factor. When an effective dose (1 mg) was divided and given in 3 separate injections at 10-minute intervals, vascular resistance in guinea pigs was only slightly increased. It has been suggested(2) that serotonin reaching the circulation from the venous side may be removed by the lungs. It is possible, therefore, that the rapid administration of a large amount of this agent raises the

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† Vismara terapeutici, Casatenovo, Brianza (Como), Italy.

TABLE I. Effects of 5-hydroxytryptamine on Vascular Resistance to Negative Pressure in Irradiated, Thrombocytopenic* Guinea Pigs.

No. of animals	Material	Dose	Route of admin.	Avg No. of Petechiae: Prior to inj.	10′ after inj.	30′ after inj.	60′ after inj.
20	saline	.2 cc	Intrav.	23 (19-26)†	21 (17-23)	24 (20-27)	20 (18-23)
25	serotonin	1 mg	Intrav.	21 (18-23)	2 (0- 5)	8 (1-12)	17 (12-21)
20		.5 "	"	26 (22-30)	7 (0-12)	9 (3-16)	29 (26-35)
18		.1 "	"	19 (17-21)	18 (16-23)	20 (17-25)	22 (18-25)
24		1 "	Intraper.	25 (21-29)	4 (0- 9)	16 (8-26)	28 (24-31)
15		.5 "	"	34 (31-38)	16 (11-28)	23 (18-32)	31 (28-34)

* Platelet counts less than 20,000/mm³. † Range of individual determinations.

TABLE II. Effects of Serotonin on Large Vessel (Tail-Snip) Bleeding of Normal and Irradiated Mice.

No. of animals: Irradiated*	Normal	Agent	Dose	Route	Avg time of bleeding (min.): Irradiated animals	Normal animals
75	25	saline	.2 cc	Intraper.	37 (32-43)†	9 (7-13)
30	10	serotonin	1 mg	"	4 (3- 7)	3 (2- 4)
36	20		.5 "	"	7 (4-10)	5 (3- 9)
50	20		.25 "	"	22 (14-36)	8 (4-14)

* Platelet count less than 40,000/mm³. † Range of individual determinations.

blood level above the threshold for pulmonary removal, thus permitting the excess to enter the arterial circulation and to exercise a generalized effect on the vascular bed. It should be noted also that the effects observed in our studies were of short duration.

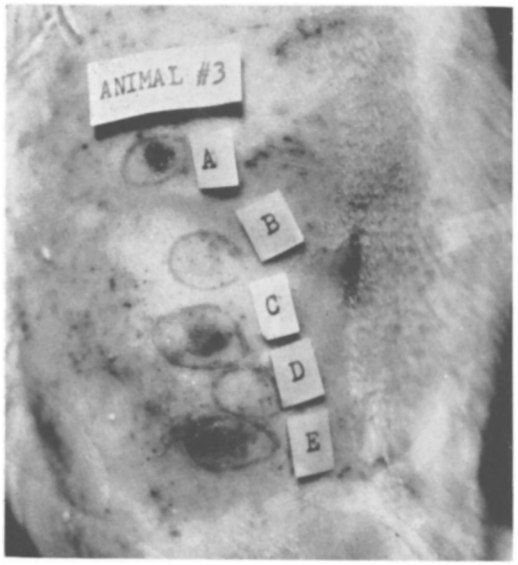

FIG. 1. Effect of intrav. serotonin admin. on vascular fragility of thrombocytopenic guinea pigs. A, C, E = Petechiae formed by negative pressure prior to inj. of serotonin. B, D = Tests 10′ following intrav. admin. of 0.5 mg serotonin.

Clinical investigations of serotonin have been confined to administration of relatively small doses(3,4). Clinical studies, which would be analogous to the animal studies reported here, have not been reported. Before effective doses and optimal modes of administration can be studied in man, the relation of hemostatic activity to toxic manifestations will have to be evaluated further. It should be noted, in this connection, that renal damage was not observed with the doses required for hemostatic effects in this study. The data presented here suggest that further clinical evaluation of serotonin as a hemostatic agent may be warranted, should the administration of higher doses prove feasible.

Summary. Synthetic serotonin (5-hydroxytryptamine-creatinine sulfate) increased vascular resistance and shortened the duration of traumatic bleeding in irradiated thrombocytopenic guinea pigs and mice, respectively. These effects were of short duration and were observed only when large doses of serotonin were given rapidly. Transient pulmonary distress was associated with the administration of effective doses to guinea pigs.

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Anaphylaxis in Guinea Pig: Improbability of Release of Serotonin in the Schultz-Dale Reaction.* (23803)

MARY ALEXANDER FINK AND CHARLES E. GARDNER

Department of Microbiology, University of Colorado Medical School, Denver, Colo.

Much knowledge concerning anaphylaxis has been gained from experimentation using the guinea pig, where it repeatedly has been shown that most symptoms of anaphylaxis are referable to release of histamine. The methods often employed antihistaminic drugs, but as pointed out by Dale(1), and Hawkins and Rosa(2), these drugs have inconsistently blocked the reaction; and their specificity is often suspect. Also, biological assay methods to measure histamine could conceivably be detecting other similarly reactive substances. These findings, together with failure to incriminate histamine in the rat and the persistence of the Schultz-Dale reaction in guinea pig tissues made refractory to histamine(3) cast doubt on the histamine theory of anaphylaxis. Other substances which might be involved in addition to histamine have been discussed by Dragstedt(4). Recent work on another species, the mouse, has demonstrated the improbability of involvement of histamine in the anaphylactic reaction; and the probability of the release of 5-hydroxytryptamine (serotonin). Evidence supporting the serotonin hypothesis includes prevention of the Schultz-Dale reaction in the presence of the highly specific serotonin antagonist, lysergic acid diethylamide (LSD),† and the demonstrably antiserotonic drug, reserpine(6). Further, Weiser(7) has shown almost 100% inhibition of generalized anaphylactic symptoms with reserpine; and Fox *et al.* with LSD(8). Conflicting reports have appeared recently regarding the role of serotonin in guinea pig anaphylaxis. Herxheimer(9) has shown that, although the guinea pig responds to an aerosol of serotonin in a manner similar to its response to histamine, and desensitization to an antigen is accompanied by a parallel tolerance to serotonin, LSD does not alter the anaphylactic reaction obtained after inhalation of antigen. During the course of the present work, however, Geiger *et al.*(10) reported that the Schultz-Dale reaction and the reaction to serotonin, but not the reaction to histamine, could be inhibited with the drugs yohimbine, bufotenine and gramine.

The purpose of this work was to determine whether or not the Schultz-Dale reaction in the sensitized guinea pig could be prevented with the serotonin antagonists LSD and brom lysergic acid diethylamide (BOL)(11). Following the publication of Geiger's work, an attempt was made to confirm his findings.

Procedure. Fully grown guinea pigs of both sexes from various commercial sources were used. They were injected intra-

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† Munoz(5) has claimed that LSD does not specifically block serotonin, but this claim was not substantiated by any reference. We have been unable to uncover any evidence that LSD in the concentration used in these studies antagonizes the action of any other known physiological substance on smooth muscle.