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Effect of Injecting Platelet Extract Intravenously.*† (20383)

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It is now fairly widely accepted that platelets do not contain any significant amount of preformed thromboplastin, but supply a factor which reacts with a plasma constituent, thromboplastinogen, to form active thromboplastin (1). Recent studies indicate that before this reaction can occur, thromboplastinogen must become activated either by contact with a foreign surface such as glass(2) or by the addition of thrombin(3). Since circulating blood probably contains no activated thromboplastinogen, the injection of a platelet extract should not cause intravascular clotting nor have any pronounced effect on the blood. The present investigation was undertaken to test the correctness of this conclusion. As a control, the effect of injecting tissue extract containing active thromboplastin was also studied.

Methods. Platelet extract from rabbit blood was prepared according to the method previously described(2). The procedure consists essentially in obtaining platelets by differential centrifugation, and then causing disintegration by repeated freezing and thawing while suspended in distilled water. This procedure is very effective for disintegrating platelets as revealed by phase contrast microscopy and by the loss of all power to bring about clot retraction. *Rabbit brain extract* was prepared by triturating fresh brain tissue, cleared of blood vessels, with distilled water, then subjecting it to freezing and thawing similar to the procedure used for platelets. The emulsion was centrifuged for 5 minutes at 1000 rpm to remove coarse particles and then was allowed to remain at room temperature for 3 hours to assure inactivation of any adenosine triphosphate(4). Both the platelet extract and the brain extract were adjusted to a sodium chloride concentration of 0.85%. The *fibrinogen concentration* was determined by the method previously described(5) except that instead of employing the Folin Ciocalteu reagent, the fibrin digested in sodium hydroxide was estimated directly by determining the optical density at 280 μ. The *prothrombin time* and prothrombin consumption time were determined by the methods previously described(5). Platelets were counted directly in a hemacytometer using 3.8% sodium citrate solution as diluent. All injections were made into the marginal vein of the rabbit ear and were given at the rate of about 3-5 cc per minute. Blood samples for analysis were taken from the central artery of the ear.

Results. When a potent extract of platelets in an amount equivalent to as high as 210%

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TABLE I. Effect of Injecting Platelet Extract Intravenously into 6 Rabbits.

Wt of rabbit (kilos)	No. of platelets inj. as extract	Calculated equivalent % of platelet in circulation	Fibrinogen conc. (mg/100 cc of plasma) Before	After platelet extract inj. 5 min.	15 min.	45 min.	4 hr
4.0	7.2×10^{10}	25	176	168	180	—	—
1.6	10.3×10^{10}	210	258	—	249	—	244
3.4	4.2×10^{10}	42	180	182	—	190	—
4.0	28.0×10^{10}	30	213	202	—	219	—
2.7	7.8×10^{10}	70	277	289	—	270	—
3.4	5.3×10^{10}	142	166	168	—	173	—

Prothrombin time in all animals remained normal (6 sec.), prothrombin consumption time of whole blood occasionally showed slight but insignificant variations. Platelet count always decreased, but rarely below lower range of normal.

Platelet extract inj. into rabbit #6 contained 234 mg of platelet solids in 12 ml of extract. Two weeks later a brain tissue extract containing 52 mg of brain solids in 12 ml of extract was inj. at same rate. The rabbit died in less than 3 min. after the end of the inj. Autopsy showed massive pulmonary embolism.

of the number of platelets in circulation was injected intravenously into rabbits, no striking systemic effects were noted, the prothrombin time and the prothrombin consumption times were not significantly altered and the fibrinogen concentration remained unchanged (Table I). Thus, the injection of a platelet extract containing 234 mg of platelet solids was entirely innocuous, yet the injection of rabbit brain extract containing 52 mg of brain solids into the same animal employing the same technic, killed the animal in 3 minutes by causing a massive pulmonary embolism. The ineffectiveness of platelet extract *in vivo* cannot be attributed to lack of potency since the material employed brought about a marked consumption of prothrombin when added to normal deplateletized rabbit plasma and decreased the clotting time of recalcified highly-centrifuged plasma when the reaction was carried out in glass test tubes (Table II).

TABLE II. Potency of Platelet Extract as Estimated by Prothrombin Consumption Time and Clotting Time of Highly-Centrifuged, Recalcified Citrated Rabbit Plasma.

	Conc. of platelet extract, % 0	1	3	10	100
Clotting time of recalcified plasma (sec.)	1600	520	180	145	125
Prothrombin consumption time 1 hr after clotting (sec.)	9	14	27	41	63

To 1 ml of citrated plasma were added 0.1 ml of platelet extract and 0.1 ml of 0.25 M $CaCl_2$. After mixing, the tube was incubated at 37°C. Platelet extract used was an aliquot of that inj. into rabbit #6 in Table I. Undiluted platelet extract (100%) contained approximately 20 mg of platelet solids/ml.

The results reported in this study clearly suggest that the products from mechanically disintegrated platelets are inert in circulating blood. This is in accord with our recent findings that the platelet factor can act only after thromboplastinogen has been activated. The present findings fail to support the theory of Brinkhous(6) that "normal plasma contains a factor necessary for thromboplastin liberation from formed elements of the blood, presumably by platelet lysis." In conformity with our previous findings(2), it is unlikely that the clotting agent in platelets is an enzyme as one of us had postulated(5). It is probable that intravascular clotting is initiated only if either thrombin or preformed thromboplastin such as occurs in tissues gains entrance into the blood stream.

Summary. The injection of a potent extract of rabbit platelets into normal rabbits fails to produce any demonstrable effect on the coagulation system and does not induce intravascular clotting.

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