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Received November 8, 1951. P.S.E.B.M., 1951, v78.

Separation, Concentration, and Transfusion of Platelets.* (19223)

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Evaluation of the therapeutic usefulness of blood platelets has been hindered by the lack of a method which permits the efficient separation and concentration of these elements physiologically intact in quantities suitable for transfusion. The method described below has met these requirements reasonably well, and may serve as a useful point of departure for others interested in this field. It has been kept sufficiently simple that no unusual technical skill or equipment are required.

Materials and methods. The method is based on the use of di-sodium ethylene diamine tetra-acetate (Sequesterene Na_2†) as anticoagulant, and the use of differential centrifugation for the separation and concentration of the platelets. One g of Sequesterene and 0.7 g of sodium chloride were dissolved in 100 cc of distilled water. Nine parts of blood were added to one part of the Sequesterene solution. Venous blood was collected from humans, and arterial or venous blood from dogs, by gravity flow through a coated 15 gauge nylon-hub electro-polished needle‡ and plastic tubing‡ into 200 ml siliconed centrifuge bottles, each containing 20 cc of the Sequesterene solution. The guinea pig blood was aspirated from the heart into a syringe containing the Sequesterene solution and transferred to the centrifuge bottles. The bottles were carefully balanced and centrifuged at 30 x g for 50 minutes at 5°C. The supernatant platelet rich plasma was then siphoned into another 200 ml siliconed glass centrifuge bottle, using siliconed glass and plastic tubing as the siphon. This plasma was centrifuged at 300 x g for 50 minutes at 5°C, and the supernatant siphoned off leaving the platelets as a loosely packed mass in the bottom of the tube. The platelets were then resuspended in an aliquot of the supernatant plasma. Resuspension was readily accomplished by aspirating and expelling the platelet-plasma suspension 3 or 4 times in a siliconed sterilized cotton plugged pipette with a tip bore of 1 mm or more. All platelet counts were done by the method of Brecher and Cronkite(1). To test the viability of the platelets, animals were usually given the equivalent of their normal total platelet content by transfusion, the volume of suspension varying from 1-2 ml in guinea pigs and 10-25 ml in dogs. Sixty-three transfusions of pooled platelets were given to guinea pigs, and 19 were given to dogs; 4 of the guinea pigs were normal, 13 were irradiated, 3 of the dogs were normal, and 3 were irradiated. The transfusions were given within one hour of the preparation of the suspension and within 4-5 hours of drawing the blood. No platelet transfusions were at-

* The opinions and assertions are those of the authors and are not to be construed as reflecting the policy or opinions of the Naval Service.

† Supplied by courtesy of the Alrose Chemical Co., Providence, R. I.

‡ Product of the Fenwal Co., Ashland, Mass.

TABLE I. Results of Platelet Transfusions on Platelet Levels of Normal and Irradiated Dogs.

Dog No.	Post irradiation day	Platelets count, 1000/mm³ Before transfusion	Platelets count, 1000/mm³ 2 hr after	Total platelets inj., × 10^{10}	Vol susp., ml
257	Normal	365	395	1.6	25
334	"	300	365	2	25
335	"	300	365	6	25
335	11	8	20	18	30
335	12	40	140	13	20
335	13	58	125	11	30
335	14	115	190	21	21
337	15	60	180	12	20
338	5	295	285	6	20
338	6	190	275	10	20
338	7	165	225	7	20.5
338	8	175	205	17	22.5
338	9	125	200	15	22.5
338	10	130	120	3*	21
338	11	53	130	8	20
338	12	30	148	28	23
338	13	70	185	46	24.5
338	14	90	235	33	25

* Clumping observed in suspension.

tempted in humans.

Results. Centrifugation of whole blood as described above resulted in packing the red cells until they occupied a volume of approximately 60% of the whole blood. The white cells, along with some platelets, were above the red cell mass as a loosely packed buffy coat. The supernatant plasma contained platelets and an occasional small lymphocyte. The platelet count of this plasma was usually double the platelet count of whole blood in the case of human and guinea pig blood. With dog blood the platelet count was the same as that of the whole blood or only slightly higher. The use of a 1.5% Sequesterene solution instead of the usual 1% solution did not increase the platelet concentration in the dog plasma. Platelets not accounted for in the supernatant plasma could, however, be recovered by resuspending the buffy coat and the top 10% of the red cell mass in plasma, and repeating the first centrifugation. These platelets were not clumped, and could be readily concentrated and resuspended. Generally, not all of the plasma could be siphoned off without disturbing the buffy coat, hence a constant loss of supernatant plasma of about 10% was encountered. All of the platelets from the supernatant could be recovered in the second centrifugation if the tubes were well balanced. The recoveries of platelets from whole blood were: 50% for dog blood, 70% for human blood, and 85% for guinea pig blood. Examination with the phase microscope of the platelets in whole blood, supernatant plasma or in the final suspension, revealed no morphologic alteration during processing. In the final suspension there was no tendency to clump or to adhere to unsiliconed glass surfaces.

In vitro the blood of irradiated thrombopenic dogs has a prolonged clotting time of 30 minutes to several hours, forms a fragile non-retractile clot, and fails to utilize prothrombin. The addition of platelets prepared with the present method caused clotting in less than 2 minutes, utilization of prothrombin and clot retraction(2). No comparable studies were made with guinea pig blood. *In vivo* 20 to 70% of the transfused platelets were present in the peripheral blood of normal as well as irradiated dogs 2 hours after intravenous transfusion. Approximately 50% of the platelets were still present 24 hours later as judged from the platelet levels observed on the following day before the next transfusion was given (Table I). In the irradiated thrombopenic dog whose platelet level was maintained at approximately 100,000 per cu mm by platelet transfusion, the coagulation defect and the hemorrhagic tendency were prevented(2).

In guinea pigs with an average weight of

TABLE II. Effects of Platelet Transfusions on Platelet Levels in Guinea Pigs.*

Platelet preparation lot and platelets per mm³ × 1000	A 4200		B 3500 (heated to 40°)			C 6300		D 7000			E 3000	F 7000	
Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Platelet count before transfusion × 1000	170	210	260	200	170	20	40	80	10	220	160	270	150
Platelet count 30' after transfusion × 1000	400	350	240	140	200	110	170	260	50	390	240	270	700

* Animal No. 1-9, irradiated, transfused with 1 cc suspension. No. 10-13, normal controls, transfused with 2 cc of suspension.

250 g the average blood volume was estimated to be 20 ml (8% of body weight). On injection, the platelets contained in 1 ml of a suspension would be redistributed in approximately 20 times the original volume. Provided that all of the platelets continued to circulate, the injection of 1 ml of a platelet suspension containing 1,000,000 per cu mm would be expected to raise the count of the recipient by 50,000 per cu mm. Table II gives the counts obtained before, and 30 minutes after, intracardiac injection of 6 platelet suspensions ("A" to "F") into 13 guinea pigs. Increases in the platelet level were observed in 9 animals and amounted to 10-80% of the calculated optimal rise. In 2 guinea pigs (No. 5 and 12) the platelet level remained unchanged and in 2 (No. 3 and 4) it dropped. Three of these guinea pigs (No. 3, 4, and 5) received a suspension ("C") which had been heated accidentally to 40°C. The 4th animal (No. 12) received a suspension ("F") which raised the platelet count of another animal (No. 13) to 80% of the optimal calculated level.

No febrile reactions to platelet transfusions occurred when sterile pyrogen free equipment and materials were used. One irradiated dog developed an extensive urticaria following the 5th of a series of transfusions.

Discussion. The tendencies of platelets to become sticky, to agglutinate rapidly, and to undergo morphologic alterations in shed blood are well known. Sequesterene Na_2 minimized these changes whereas other anticoagulants including citrate and ACD§ were ineffective. In unpublished experiments, blood decalcified by passage through a cation exchange resin column at room temperature was also found unsatisfactory for the preparation of platelet suspensions. Such blood remained fluid, but most of the platelets were retained in the column. The resins used were a sulphonic cation exchange resin, Dowex 50, and a carboxylic cation exchange resin, Amberlite IRC 50, both on the sodium cycle, buffered to pH 7.2. Amberlite IRC 50 when cooled to 1°C did not remove platelets from blood precooled to 7-9°C. Recovery of platelets from this blood, however, was not practical because of stickiness and rapid irreversible clumping. This was possibly due to the very slow flow rate at low temperatures which may have allowed changes in the platelets to occur before the blood reached the column and calcium was removed.

In the absence of adequate *in vitro* technics for determining platelet viability it has been necessary to evaluate preparations *in vivo*. A considerable proportion of the transfused platelets appeared to maintain their functional integrity in that they continued to circulate for 24-48 hours, and reversed the coagulation defect and hemorrhagic tendency in irradiated thrombopenic dogs(2). In the normal animal part of the platelet rise following transfusion might be accounted for by a rapid release of the animal's own platelets into the circulation. In the irradiated animal which produces no platelets the rise in circulating platelets can be accounted for only by the continued circu-

§ Solution of tri-sodium citrate, citric acid and dextrose, as specified in Minimum Requirements for Citrated Whole Blood, Biologics Control Laboratory, National Institutes of Health.

lation of transfused platelets. The marked variation in the rise of circulating platelets in different animals given transfusions from a single suspension, or from a different suspension prepared in identical fashion at another time, cannot be explained at present.

When complexed with calcium, Sequesterene is relatively non-toxic(3). Proescher has shown the usefulness of Sequesterene as an anticoagulant for humans(4). In our limited experience Sequesterene has been non-toxic as well as non-pyrogenic. Although large numbers of platelets were removed from the circulation of the experimental animals within 30 minutes to 2 hours of transfusion, no evidence of massive thrombosis or embolism has been found.

Summary. (1) A simple method is presented for the efficient separation and concentration of platelets from human, dog, and guinea pig blood in quantities sufficient for transfusion. (2) This method depends upon the use of Sequesterene Na_2 as an anticoagulant, which prevents the development of platelet stickiness and clumping in shed blood. (3) Platelets prepared by this method and transfused into the thrombopenic, irradiated dog remain in the circulation, reverse the coagulation defect, and prevent hemorrhage.

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Received November 9, 1951. P.S.E.B.M., 1951, v78.

γ-Aminobutyric Acid Content and Glutamic Decarboxylase Activity in Developing Mouse Brain. (19224)

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γ-Aminobutyric acid is found in the free form in large amounts in brain and spinal cord(1-4). This amino acid and the enzyme which catalyzes its formation from L-glutamic acid by alpha decarboxylation are present in uniquely high concentrations in the tissue of the central nervous system(5,6). The enzyme requires pyridoxal phosphate as a coenzyme, which can be synthesized by brain from adenosinetriphosphate and pyridoxal(5,6). When rats were fed a diet deficient in pyridoxine there was a decrease of approximately 50% in the degree of saturation of apoenzyme with coenzyme, but the content of apoenzyme was normal(7). Refeeding pyridoxine to rats previously made deficient resulted in a return of the activity to a normal level(7). In the present experiments a study was made of the content of γ-aminobutyric acid and the activity of glutamic decarboxylase in the brains of mice at various stages of development in an effort to delineate the role of this system in brain metabolism. Some correlative observations were made on the nervous tissue of cats, rats, dogs, and rabbits.

Experimental. Animals, preparation of tissues, and analyses. All mice, rats, and rabbits employed were killed by dislocation of the cervical vertebrae. The cat and dogs were killed by intravenous nembutal. Whole mouse and rat brains were used for analysis. Alcoholic extracts of the tissues were analyzed for the content of γ-aminobutyric acid by a paper chromatographic method(3) and the maximal potential glutamic decarboxylase activity was determined by a manometric pro-

* This investigation was supported by a research grant from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, and the Charles F. Kettering Foundation.