tern previously reported.

Discussion. Rigidly controlled experiments suggest that within definite limits 1-ethanesulfonyl-4-ethyl-piperazine administered intravenously is effective in prolonging the survival time and decreasing the mortality in dogs subjected to extreme hemorrhagic shock. The protective action of the drug occurred when it was administered in a dosage of 50 to 100 mg/kg within the first 30 minutes of hypotension. No protection was evident when hypotension was present for 90 minutes before the drug was injected.

The procedures used varied somewhat from others previously employed in testing a drug for the treatment of hemorrhagic shock(5). Preliminary experiments suggested that a 1½ to 2 hour period of hypotension did not consistently produce a state of irreversible shock. The period of hypotension was therefore prolonged to as much as 3 hours with an average of 2 hours and 30 minutes. To obviate variations of seasons of the year, weather, and technic, all experiments were done on paired dogs, so that a control was run simultaneously with the experimental animal.

The pharmacodynamic actions and toxicity of 1-ethanesulfonyl-4-ethyl-piperazine were not determined in these experiments although it was determined that larger doses could be injected much more rapidly in normotensive dogs. Thus, a definitely increased toxicity was present in some of the hypotensive animals. Evidence of this was the pronounced drop in blood pressure which resulted soon after administration of the drug for which compensation did not occur for approximately 30 minutes (Fig. 2). In several animals compensation never occurred and death ensued rapidly (Dog 6, Table I). During injection of the drug a rather marked increase in respiratory rate and depth usually was evident.

Summary. 1. When dogs which had been subjected to severe hemorrhage were treated within 30 minutes with 50 to 100 mg/kg of 1-ethanesulfonyl-4-ethyl-piperazine administered intravenously, survival time was prolonged or mortality decreased in comparison to untreated control animals. 2. No protective action was evident when treatment was withheld until there had been 90 minutes of hypotension. 3. The piperazine compound is definitely toxic and was considered responsible for death of 3 of 30 dogs in which it was used for treatment. 4. The drug imposed a decided hypotensive factor on the acute 40 mm Hg state produced deliberately by controlled hemorrhage.

Dr. William Young, Chief of Surgery at the Veterans Administration Hospital, Madison, helped institute work on this project.

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Platelets: VII. Shortened "Platelet Survival Time" and Development of Platelet Agglutinins Following Multiple Platelet Transfusions.* (19579)

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In the course of studies on the fate of platelets injected into patients with various types of thrombocytopenia(1,2), it was observed that the survival time became shorter

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or, more strictly, the rate of disappearance of platelets from the circulation was accelerated when the patients received repeated transfusions of platelet-rich polycythemic blood or suspensions of isolated platelets. At the same time, it was discovered that platelet agglutinins developed occasionally.

Materials and methods. Because of various abnormal and possibly immuno-humoral factors in idiopathic thrombocytopenic purpura (2-5), patients with this disease were excluded from the present series. Serial determinations of platelet survival time were conducted in 4 cases, 2 with hypoplastic and 2 with aplastic anemia. These patients presented severe thrombocytopenia requiring repeated transfusions of platelet-rich polycythemic blood or of isolated platelets for the control of bleeding. Simultaneously, the serum was studied for platelet agglutinating properties in these 4 patients and in 11 additional patients who had received multiple transfusions of blood over a period of months.

(a) *Technic for the determination of survival time of injected platelets.* Transfusions of platelet-rich polycythemic blood or plasma were given by the direct technic previously described(2). The blood or plasma administered was of homologous group and type and compatible as determined by cross-matching in albumin medium. Moreover, blood was given only after a mixture of inactivated, decalcified and deprothrombinized serum of the recipient and saline suspension of platelets from the donor had failed to show clumping after 12 hours of incubation at room temperature in Silicone-coated tubes, thus presumably indicating the compatibility of platelets. Clot retraction, bleeding time, capillary fragility and utilization of prothrombin during the coagulation of blood were followed in each patient with the procedures previously described(2). Platelet count was determined by both the indirect method of Dameshek(6) and by a direct method(7). Values were considered significant only if in relative agreement. The figures shown are those obtained with the indirect method.

(b) *Detection of platelet agglutinins in serum.* 1) *Preparation of platelet suspensions.* Platelet suspensions were freshly prepared and all procedures were carried out in Silicone-coated glassware. After their separation from plasma components and the various cellular elements of the blood, platelets are reasonably well preserved even when in contact with foreign surfaces such as glass. Nevertheless, the use of non-wettable surfaces decreases the loss or irreversible agglutination of platelets during their separation. We have also noticed that when ordinary glassware is used human platelets may at times become less agglutinable by specific anti-human platelet sera obtained from animals. Blood was collected from a donor's vein by the 2-syringe technic(8), through Silicone-coated needles in Silicone-coated syringes; it was then transferred to 15 ml Silicone-coated test tubes containing 1/10 volume of 0.2 M sodium citrate. More recently, 1% Sequestrene-Na_2§ in 0.7% sodium chloride solution has been used routinely. The blood was spun in a refrigerated centrifuge at 500 r.p.m. for 15 minutes, the plasma separated and allowed to stand for one hour at room temperature, and finally centrifuged for 10 minutes at 1,000 r.p.m. to separate most of the remaining red and white cells.‖ The platelets were then collected at the bottom of the tube by centrifuging at 2,000 r.p.m. for 20 minutes, and resuspended in a small volume of saline solution containing 0.2% sodium acetate. The platelet suspension was then diluted with saline-acetate solution to obtain a final count of approximately 500,000 platelets/cu mm by a direct method(7). The suspension was considered satisfactory only when

§ Disodium ethylenediaminotetraacetate dihydrate, obtainable from the Akrose Chemical Co., Providence.

‖ Addition of 1/10 volume of 2% sol. of Tween-80 (Rohm and Haas, Philadelphia) to plasma prior to centrifugation allows resuspension of platelets in saline with minimum loss due to clumping. Following Minor and Burnett(9) we have now substituted Triton W-1339 for Tween-80 with even more satisfactory results and use this procedure routinely. The minute amount of the surface active agent which remains with platelets does not appear to influence their agglutinability nor cause spontaneous clumping. A Sequestrene-Triton technic, to be described, is now used routinely in this Laboratory for the preparation of large number of platelets for use in humans.

free of clumping on microscopic reading and containing less than one red or white cell per high power microscopic field. 2) *Preparation of serum for agglutination studies.* Blood was collected from an antecubital vein, allowed to clot at 37°C for one hour, and the serum separated by centrifugation. After incubation at 56°C for 30 minutes to inactivate complement, serum was divided in small aliquots and these kept frozen at —20°C until used (20 days to 4 months). Since we have previously shown that addition of platelets to serum containing prothrombin, calcium and other coagulation factors will result in non-specific agglutination of platelets, the serum was decalcified and/or deprothrombinized by the technics previously described(10). Dilutions of serum were prepared with phosphate-saline buffer (pH 7). 3) *Agglutination test.* The blood type and group of the patient under investigation were first determined and platelets from a compatible donor prepared. 0.1 ml of platelet suspension and 0.1 ml of serum at various dilutions were transferred to 8 x 50 mm serology tubes coated with Silicone by means of Silicone-coated pipettes, the mixture allowed to stand at room temperature for one hour and kept overnight at 4°C. Alternatively, plasma rich in platelets, as obtained by low speed centrifugation (10 minutes at 500 r.p.m.) of blood collected in Sequestrene-Na_2, could be used, provided the serum had been inactivated, decalcified and/or deprothrombinized and the platelet count adjusted to 500,000/cu mm diluting, if necessary, with platelet-poor plasma obtained by prolonged centrifugation at high speed. Two control test tubes were also prepared: one containing 0.1 ml of buffered saline and 0.1 ml of platelet suspension and the other a mixture of 0.1 ml of platelet suspension and 0.1 ml of normal human serum kept frozen at —20°C for 20 days and inactivated, decalcified and/or deprothrombinized prior to use. After overnight incubation, all test tubes were returned to room temperature for 30 minutes and the platelets (which had settled to the bottom) resuspended by gently tapping the bottom of the tube. More vigorous tapping of the tube, although reducing the size of the clumps, failed, in all cases, to cause complete resuspension of the agglutinated platelets. Presence of clumping was read macroscopically, over a concave lens giving a X3 enlargement under a strong light, and microscopically, under high dry objective. Small drops of Silicone were observed in the field at times, but they did not interfere with the reading. All experiments were run in duplicate and, in order to minimize the human error of the technic, the results were read by the same two observers, and only the results in agreement accepted as valid. The procedure described has been currently employed during the past year for the search of antiplatelet substances in serum of patients with idiopathic thrombocytopenic purpura and secondary thrombocytopenia. A series of 112 normal sera has been completed thus far and negative results have been consistently observed provided the serum has been kept frozen at —20°C for 20 days and inactivated, decalcified and/or deprothrombinized prior to use. The mechanism of the ability of fresh normal sera to agglutinate platelets will be discussed in another communication(11). By this method, also, platelet agglutinin have been demonstrated in a number of cases of isopathic thrombocytopenic purpura.

Results. a) *Survival time of injected platelets after repeated platelet transfusions.* The 4 patients in whom the rate of disappearance of platelets injected by direct transfusion of platelet-rich polycythemic blood was studied at intervals of time had received frequent transfusions of fresh blood in plastic bags, isolated platelets and polycythemic blood by direct transfusion(2). In 3 of these patients the survival time of injected platelets became progressively shorter and, simultaneously, the beneficial effect of platelet transfusions on the bleeding manifestations, bleeding time, capillary fragility and coagulation process became less pronounced. A typical example is shown in Fig. 1. The platelet survival time of this patient, a case of hypoplastic anemia, was of approximately 4 to 5 days, on 5/12/1950. On 5/29/1950 he received a transfusion of 350 billions platelets suspended in plasma and on 6/13/1950 the platelet survival time had decreased to 3 days. Two transfusions of fresh blood collected by gravity and admin-

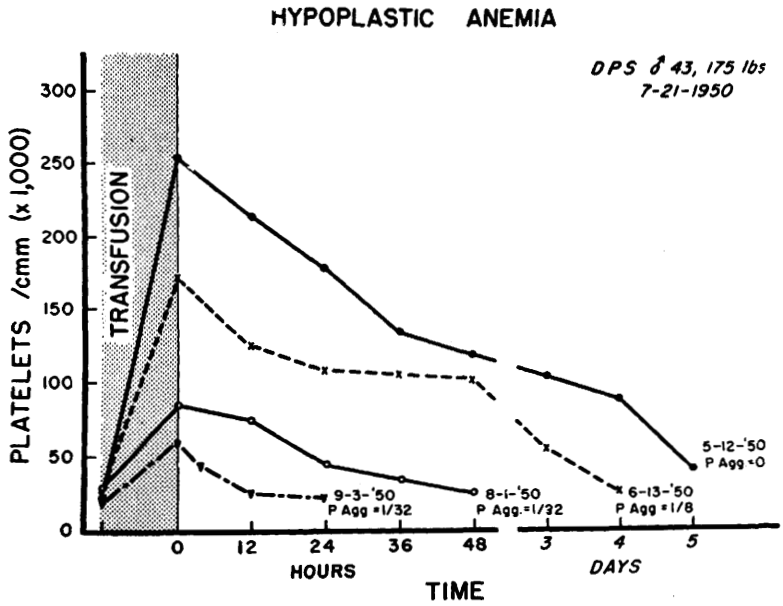

FIG. 1. Shortened survival time of injected platelets and development of a platelet agglutinin in serum of one patient receiving multiple platelet transfusions. (Shaded area represents time necessary to complete the platelet transfusions: 15 to 45 min).

istered with Silicone-coated bottles, connecting tubes and needles were given on 6/27/1950 and 7/12/1950. On 8/1/1950 the platelet survival time was reduced to approximately 24 hours and most thrombocytes had been quickly removed from the circulation. After another transfusion of 280 billions platelets resuspended in plasma on 8/24/1950, platelets injected on 9/3/1950 were very quickly removed from the circulation. At the same time, no improvement of petechial bleeding and epistaxes followed the last two platelet transfusions. The increased rate of utilization or destruction of platelets was also indicated by the extent of rise in the platelet level immediately after platelet transfusion which fell progressively shorter of the figure to be expected on the basis of the calculated number of thrombocytes injected and the patient's blood volume. b) *Development of acquired platelet agglutinins in patients receiving multiple platelet transfusions.* The serum of patient of Fig. 1 was found to have developed a platelet agglutinin, with a titer which rose progressively to 1/32, and remained until the patient's death from cerebral hemorrhage, being consistently demonstrable in several tests. The agglutinin did not seem capable of affecting the functional ability of normal platelets, as demonstrated by the following tests. Normal platelet-rich native human plasma was obtained by centrifugation at 500 r.p.m. at 4°C for 10 minutes of blood collected in Silicone. 0.3 and 0.5 of the patient's native serum were added to 0.7 and 0.5 ml of compatible native plasma respectively. In another series, normal native serum was added to normal native plasma in equal proportions. All mixtures were allowed to clot at 37°C and the retraction of the clot and the prothrombin activity of serum determined by methods previously described(8,12). No differences were observed between mixtures containing normal serum and those containing serum with the platelet agglutinating property.

Agglutinins against platelets were not found in the sera of the other 2 patients who had developed increased "resistance" to transfused platelets. In view of these findings, search for platelet agglutinins was conducted in the serum of 11 patients who had received multiple transfusions of blood within a few months. They included 2 cases with bleeding peptic ulcer, 2 cases with prostatic carcinoma and severe urinary bleeding, 3 cases of acute lymphocytic leukemia, 2 cases of Mediter-

ranean anemia, and 2 cases of disseminated carcinomatosis with myelophtisic anemia. Six patients presented a normal or elevated platelet count, 4 a marked thrombocytopenia. One developed thrombocytopenia following multiple transfusions, as will be mentioned later. These patients received blood collected with the conventional technic in ACD solution and glass vacuum containers. This blood contained few platelets, as demonstrated by the result of direct count and its poor clot retraction on recalcification. Large amounts of platelet products were presumably present, however, since there was excellent utilization of prothrombin following recalcification and clotting of this blood.

A platelet agglutinin (titer 1/8), demonstrable consistently in tests taken at various intervals of time, was found in the serum of one of these patients, a case of bleeding peptic ulcer who had received 12 blood transfusions of fresh blood over a period of 45 days and whose serum, at the time of admission, had not shown platelet agglutinating activity. It dropped to a 1/4 titer after 3 months during which the patient received no blood transfusions and was not detectable 6 months later. The platelet level of this patient had decreased from 850000/cu mm to 175000/cu mm and returned to values of 450000/cu mm following the disappearance of the platelet agglutinin in the patient's serum. The platelet survival time was found to be 2 days, as against the 4 to 6 days of most cases of secondary thrombocytopenia. The bone marrow did not present appreciable alterations in number or in activity of the megakaryocytes. Platelet count and platelet survival time returned to normal, following the disappearance of the agglutinin in the patient's serum.

Discussion. These observations indicate that, as patients with "secondary" or "amegakaryocytic" thrombocytopenia or even with normal platelet count are given repeated transfusions of platelets, the survival time of thrombocytes becomes progressively shortened and the beneficial influence on the bleeding manifestations less pronounced. After several platelet transfusions, the survival time of platelets may become as short as observed in idiopathic thrombocytopenic purpura, a condition in which the existence of platelet destructive mechanisms has been postulated (2-5). This finding suggests that mechanisms for platelet destruction have developed in some of these patients.

The pathogenetic mechanism involved is difficult to interpret, however. It should not be forgotten that changes in the course of the fundamental disease could be responsible for the acquired state of "resistance" to transfusion of platelets. The occasional discovery of platelet agglutinins in the serum of such patients suggests that, in particular cases, the development of "refractoriness" to the beneficial effect of platelet transfusions on the hemostatic mechanism might be due to isoimmunization against platelets or their products. Products of platelet disintegration must obviously possess antigenic properties, since the development of "refractoriness" to platelets and of a platelet agglutinin in a patient receiving blood with few intact thrombocytes was observed. The possibility cannot be excluded that iso-immunization might have been due to the repeated administration of incompatible platelets. Although we have collected some evidence for the existence of distinct platelet groups, observations of this type are handicapped by serious technical obstacles and require further careful evaluation. It is possible that these findings might be indicative of the mechanism through which thrombocytopenia develops in idiopathic thrombocytopenia purpura, or, at least, in some of these cases(13).

The cause of the decreased platelet survival in patients who had received multiple transfusions but failed to show any platelet agglutinin or platelet destroying agent in their serum remains obscure. At least part of these negative results could be due to the inadequacy of the technics presently available for the detection of anti-platelet agents.

Summary. 1. The rate of platelet disappearance or platelet survival time was followed at regular intervals in 4 patients with hypoplastic or aplastic anemia receiving repeated transfusions of platelet-rich polycythemic blood or of isolated platelets as supportive therapy. As transfusions were repeated. in 3 of these patients, a) survival time of platelets be-

came progressively shorter, b) administration of platelets was less and less effective in the control of the bleeding manifestations. In one patient a platelet agglutinin was detected in the serum. 2. Search for platelet agglutinins was conducted in the serum of 11 patients who had received multiple blood transfusions over a period of months. A low titer platelet agglutinin was detected in one case. This patient developed mild thrombocytopenia and exhibited shortened platelet survival time. 3. These findings suggest that "refractoriness" to the therapeutic effect of the administration of platelets may develop in patients receiving multiple platelet or blood transfusions. This may be due to the development of antiplatelet substances in the serum, at least in individual cases.

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Plasma Proteins in Venous and Cord Blood at Delivery Following Uncomplicated and Complicated Pregnancies. (19580)

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Protein metabolism via the human hemochorial placenta is still incompletely understood. The literature provides few determinations of the protein components in plasma from venous and cord blood samples of women at the end of uncomplicated and complicated pregnancies.

Preceding reports(1-5) have given electrophoretically determined plasma protein values for normal non-pregnant women, for women during and following uncomplicated pregnancy, and for women whose pregnancies were complicated by toxemias, diabetes, abruptio placentae, and hepatic diseases. Data for each subject who participated in the electrophoretic studies are being published in detail (6). The results obtained are in accord with those of other investigators and show that alterations of plasma protein *do* occur in uncomplicated and in complicated pregnancy. Gutman(7) has reviewed the difficulties of interpreting plasma protein levels in health and disease.

Materials and methods. Proteins were determined in the plasma of maternal venous blood obtained at or less than one hour preceding delivery and in the plasma of cord blood. Paired samples of maternal and cord blood were obtained from 11 women whose pregnancies were uncomplicated and from 21 women whose pregnancies were complicated by disease. Samples of venous blood were obtained by withdrawing 20 ml of blood from a vein in the antecubital fossa and discharging