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In vivo Transfer of a Thrombopoietic Factor.* (26874)

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The fact that the platelet count remains remarkably constant for months(1) although survival time of individual platelets is less than 2 weeks(2,3,4,5,6) suggests that there is a regulatory system or thrombocytostatic mechanism. Regulation implies feedback control in which unbalance acts as a stimulus to restore balance. According to this hypothesis, thrombocytopenia induced in an animal should stimulate thrombopoietic† activity to compensate for decreased platelet concentrations in the circulation.

Craddock *et al.*(7) and Matter and associates(8) reported increased thrombopoietic activity in dogs and rats, respectively, 4 days after production of severe thrombocytopenia by thrombopheresis. Both groups assumed that the thrombocytopenia was the sole stimulus for the thrombopoietic activity. However, recent unpublished experiments of the author showed that relatively moderate thrombocytopenia induced in rabbits by thrombopheresis resulted in pronounced thrombocytosis. Control experiments involving anesthesia and sham operations also produced very marked increases in platelet counts 3 to 4 days later. Because results were variable, the contribution of thrombocytopenia alone as a thrombopoietic stimulus was not amenable to quantitative analysis when thrombocytopenia was produced in rabbits by thrombopheresis.

These results indicated the importance of minimizing stress and suggested that a thrombopoietic factor in blood plasma might be demonstrable by intravenous transfer of plasma from thrombocytopenic donors to unstressed recipients. Provided that transfer of normal plasma was ineffective, a rise in platelet count could be interpreted as a response to a thrombopoietic factor induced by the thrombocytopenia.

Methods. Female albino rabbits (Blue Spruce) were used. Platelet counts were taken by puncture of an ear vein, using the direct method of Brecher and Cronkite(9). Although duplicate counts were not taken routinely, critical changes were often verified by a second technician. *A. Daily variations in circulating platelets.* Platelet counts of 9 rabbits were taken for 4 consecutive days and again on the seventh day. Three of the rabbits had been splenectomized more than a month prior to tests, but platelet levels had returned to pre-operative values within a week. *B. Transfer of plasma from normal donors to normal recipients.* Plasma was obtained from rabbits which showed steady platelet levels for at least one week prior to sacrifice. Blood was collected by heart puncture, performed under Nembutal anesthesia. Each 7.5 ml was put in a Becton and Dickson 3200 KA tube which provides a large excess of heparin. Within 2 hours of bleeding, 25 ml plasma were injected intravenously into each of 15 unanesthetized recipients, 4 of

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† It is recognized that the proper term is thrombocytopoietic but for convenience it is contracted to thrombopoietic.

TABLE I. Production of Thrombocytopenia in Rabbits and Effect of Injecting Their Plasma on Platelet Count of Normal Recipients.

Donor			Recipient			
				Platelet count, 10^3/mm^3		
Treatment	Interval until bled	Platelet count when bled, 10^3/mm^3	Dose, ml/kg	Day 0	Day 4	Increase, %
Myleran, 48 mg/kg	12 days	90	8.1	500	690	138
			7.8	375	460	123
Myleran, 26 mg/kg	43 "	165	8.1	325	710	218
			8.1	405	640	157
APS	20 hr	125	6.8	475	740	156
"	20 "	175	6.6	390	760	195
"	2 "	215	6.0	385	1,035	269
"	3 "	10	1.9*	445	580	131
"	3 "	—	6.6	340	710	209
			5.2	415	765	184
			2.7*	605	1,015	167
"	4 "	—	6.6	720	735	102
"	4½ "	160	6.0	480	860	179
"	3 "	—	7.4*	500	630	126
			8.1*	705	940	133
Mean ± S.D.				471 ± 121		166 ± 44

* Splenectomized recipients.

Brackets indicate that donor plasma was pooled or that several recipients received same donor plasma.

which had been splenectomized previously and all of which had demonstrated normal and constant control platelet levels for at least one week prior to injection. Doses varied from 5.9 to 8.1 ml donor plasma per kg recipient body weight. *C. Transfer of plasma from thrombocytopenic donors to normal recipients.* Two animals were made thrombocytopenic by administration of 1.4-dimethane sulphonyloxybutane (Myleran, Burroughs Wellcome Co.), and 8 were given anti-platelet serum (Table I). Myleran was homogenized with peanut oil in a concentration of 100 mg/ml and administered subcutaneously. Anti-rabbit platelet serum was prepared in dogs by a technic described by Elliott and Whipple(10), with slight alteration of antigen injection schedule. It was not adsorbed with erythrocytes or leukocytes. After intravenous injection of 5-8 ml anti-platelet serum into rabbits, the platelet counts fell to a few thousand per cu mm within a few minutes. The animals were bled between 2 and 24 hours later when platelet counts ranged from 10,000 to 215,000 per cu mm. Plasma was prepared and 25 ml injected in the same manner as normal plasma into 11 normal and 2 splenectomized recipients. Two additional splenectomized recipients received 10 ml.

Results. A. *Daily variations in circulating platelet count* (Curve A, Fig. 1). With rare exceptions, the platelet counts of healthy rabbits which had been in the laboratory for more than 10 days were between 350,000 and 700,000/cu mm and were quite constant. For the 9 animals subjected to consecutive counts, average platelet count was 514,000 ± S.D. 75,000/cu mm on day zero. On subsequent days, the group average platelet count was within 5% of this initial average, and maximum standard deviation for any day was 18%. Data for days 4, 5 and 6 of curve B, Fig. 1, were obtained by respectively comparing the counts on days 3, 2 and 1 with that on day 7.

B. *Transfer of plasma from normal donors to normal recipients* (Curve B, Fig. 1). Three days following transfer, the group average platelet levels were 88% ± S.D. 25% of average control value of 543,000 ± S.D. 121,000 platelets/cu mm blood. This was a significant decrease compared to the variations found in control Group A ($t = 2.205$;

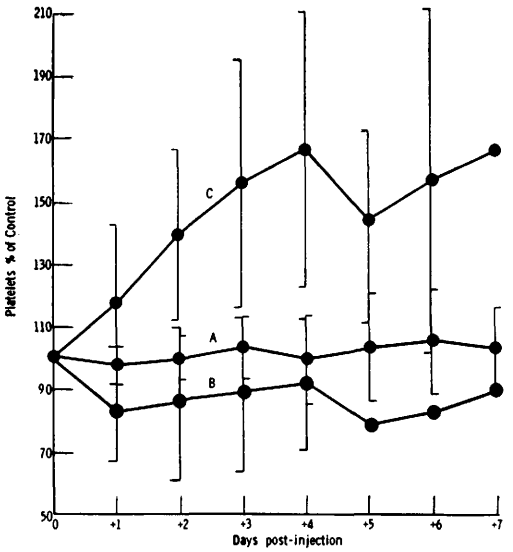

FIG. 1. Effect of plasma injections on avg platelet count of rabbits. (A) 9 uninj. rabbits; (B) 15 rabbits given plasma from normal rabbits on day 0; (C) 15 rabbits given plasma from thrombocytopenic rabbits on day 0. Brackets indicate stand. dev.

$0.05>p>0.02$. A significant decrease was not observed on the fourth day following transfer ($t = 1.29$; $0.3>p>0.2$). No difference was noted in the response of splenectomized and non-splenectomized recipients.

C. *Transfer of plasma from thrombocytopenic donors to normal recipients* (Table I; Curve C, Fig. 1). A marked increase in platelet count was observed. Comparison with the uninjected group (A) and the group receiving normal plasma (B) on the third and fourth days following transfer revealed highly significant differences ($p<<0.001$).

Discussion. Injection of plasma from rabbits made thrombocytopenic by administration of Myleran or antiplatelet serum into homologous species recipients, whether splenectomized or not, causes a marked rise in the platelet count. The duration of the response and 4-day interval between transfer and peak response suggest that the plasma stimulates production of new platelets rather than mobilization of sequestered platelets. Further experiments will be necessary to substantiate this belief. It is of interest that active plasma was obtained as early as 2 hours following production of acute thrombocytopenia by anti-platelet serum as well as after slow development of thrombocytopenia following Myleran injection. Normal plasma does not induce thrombocytosis; on the contrary, there is evidence of a significant decrease in platelet levels 3 days following injection, but not on the fourth day.‡

Other recent investigations have demonstrated *in vivo* transfer of a thrombopoietic factor. Keleman *et al.*(11) reported that injection of serum from thrombocythemic and some thrombocytopenic patients induced thrombocytosis of several days' duration in mice. Results, however, were variable because of the uncontrolled nature of the source. Injection of normal serum lowered the platelet count, and the effect of active test serum was counteracted by mixing it with normal human serum prior to injection.

Schulman *et al.*(12) observed that an unusual splenectomized patient with congenital persistent thrombocytopenia consistently showed thrombocytosis for more than 2 weeks following injection of normal plasma. They concluded that a factor in normal human plasma is required for platelet production, and that thrombocytopenia results from its deficiency.

Normal plasma or serum appears both to stimulate and inhibit thrombopoiesis, and regulation of platelet count may be governed by a balance between these actions. The stimulating activity can be demonstrated either when the recipient is deficient in thrombopoietic factor, as in Schulman's patient, or when a normal recipient receives plasma from thrombocythemic or thrombocytopenic donors. It remains to be determined

‡ In preparation for further studies on thrombopoietin, Dr. Marjorie Zucker injected 17 additional rabbits with 25 ml plasma from rabbits made thrombocytopenic with antiplatelet serum. The average platelet count 4 days later was 121% ± S.D. 40% of the control value. This was significantly different ($p<0.02$) from the platelet count 4 days after injection of normal plasma. The reason that the results were less striking than Dr. Spector's is not known. Odell and McDonald (Fed. Proc., 1961, v20, 51) have also reported transfer of thrombopoietin. Injection of serum from bled rats into normal rats elevated platelet counts to 160% of control value in 5 days.

whether the thrombopoietic effect of plasma from such donors is the result of an increased concentration of a stimulating factor or decreased concentration of an inhibitory material.

Summary. Injection of plasma from rabbit donors made thrombocytopenic by Myleran or anti-platelet serum into 15 unstressed rabbit recipients raised platelet counts to 166% of control levels 4 days following transfer. These results are highly significant ($p<0.001$) when compared to the mild thrombocytopenia produced in 15 rabbits receiving plasma from normal donors or when compared to the daily variations in platelet levels of 9 uninjected rabbits. The response of normal and splenectomized recipients was similar. These results suggest that plasma contains stimulatory and inhibitory factors which regulate platelet levels.

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Comparative Reactivities of Non-fibrous and Fibrous Forms of Desoxyribonucleoprotein with Lupus Erythematosus Serum.* (26875)

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Numerous investigators have reported that factors in the sera of patients with disseminated lupus erythematosus (LE) may react with whole cells(1), isolated nuclei(2,3), desoxyribonucleic acid (DNA)(3,4,5), desoxyribonucleoprotein (DNP)(4,6,7,8), and histone(9). It has been demonstrated that there are separate components of LE sera that react with each of the "antigens". This reactivity has been demonstrated by a variety of immunologic technics including complement fixation, latex agglutination, hemagglutination, precipitation, and fluorescence microscopy. The so-called "LE factor" is reactive only with DNP and not with isolated histone or DNA(8). In detecting the anti-DNP activity of LE sera, fibrous preparations of DNP have been utilized. These forms of DNP dissociate into DNA and histone in the molar NaCl utilized in preparation, and it is not certain that the re-association product has the identical spatial association of histone and DNA present in DNP *in vivo*. Among the numerous preparations of DNP described in the literature, only one does not dissociate in molar salt solution(10, 11). No immunologic investigations are on record, so far as we are aware, utilizing non-fibrous DNP in the role of antigenic component. The purpose of the present investigation was to determine the reactivities of non-fibrous and fibrous DNP with LE sera

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