Table II. Effect of glycogen-treatment on survival and endogenous spleen colony-formation in irradiated mice

Treatment	Survival dose of X-irradiation			No. of colonies per spleen (600 R-irradiated)
	600 R	650 R	700 R	
Control	9/25 (36%)	2/15 (13%)	2/15 (13%)	1.4 ± 0.8
Glycogen-treated[a]	13/25 (54%)	2/15 (13%)	1/15 (7%)	4.8 ± 1.3

[a] Mice were injected with 10 mg glycogen i.v. 24 h prior to X-irradiation.

able to a functional blockade of RES in the first step and to the subsequent enhancement of hemopoietic recovery. The latter has also been shown to be the result of the enhancement of the recovery as well as of the survival of hemopoietic stem cells possibly through humoral factor(s) and direct control by fortified RES[5,6]. Again, it is suggested that reinforcement of RES by blockading with particulate materials plays an important role in controling the parenchymal cells to maintain their normal function[8].

The data presented herein provides evidence which supports the hypothesis that any foreign particles which will be phagocytized by RE cells, such as macrophages, can be used as an effective radioprotectant only if they are not toxic to the animals.

One finding to be noted is that although glycogen did not protect mice effectively from radiation lethality, it also had a slightly but significantly favorable effect on the hemopoietic recovery as manifested by an increase in the number of endogenous spleen colonies. It seems, therefore, that any substance can stimulate RES non-specifically, when phagocytized, to release some factor(s) such as interferon[9] and colony-stimulating factor[10] as well as to provide a favorable environment. The degree of such effects is likely to depend on the size and dose of the particulate materials and on the persistence of them in the RES[11].

Zusammenfassung. Die i.v. Injection von Latexpartikeln an Mäusen, 24 h vor einer Bestrahlung mit 650 oder 700 R, bietet einen bemerkenswerten Strahlenschutz. Es wird mit Hilfe der endogenen Koloniebildungsmethode auch nachgewiesen, dass die Strahlenresistenz und/oder die Regeneration der hämatopoetischen Stammzellen durch die reticulo-histiocytäre Systemblockade gesteigert worden ist.

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Interaction of Thrombasthenic Platelets with Subendothelium: Normal Adhesion, Absent Aggregation

Adhesion of blood platelets to extracellular connective tissue represents the first observable step in hemostasis and thrombosis. Subsequent to adhesion, platelet aggregation results in the formation of a hemostatic plug or a mural platelet thrombus. Baumgartner[1] has recently developed a perfusion chamber for studying the interaction of platelets in anticoagulated whole blood with the subendothelial surface of rabbit aorta under controlled flow conditions that are similar to those in arteries. Platelet adhesion to subendothelium was significantly reduced, compared with control values in von Willebrand's disease[2] and the Bernard-Soulier syndrome[3]. A defect in platelet adhesion probably accounts for the prolonged bleeding time in patients with these disorders.

In this communication we report our findings on platelet interaction with subendothelium in the perfusion chamber using blood from 2 subjects with classical Glanzmann's thrombasthenia, a hemorrhagic diathesis characterized by prolonged bleeding time, absent platelet aggregation and markedly impaired clot retraction[4]. Previous investigations suggested that the adhesion of platelets in platelet-rich plasma to connective tissue is normal in thrombasthenia[5–7].

Materials and methods. Clinical and laboratory findings of the 2 patients M.C. and M.M. (the latter studied through the courtesy of Dr. Marjorie B. Zucker) have been described previously[8,9]. All 9 control subjects (ages 25–44) showed second phase aggregation with epinephrine in platelet-rich plasma on the day studied. Patients and controls were asked not to take any medication for 1 week prior to the experiment.

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Platelet interaction with subendothelium in thrombasthenia

	Hematocrit	Platelet count ($\times 10^{-3}/\mu l$)	Platelet interaction with subendothelium		
			Platelet contact (%)	Platelet adhesion (%)	Platelet thrombi (%)
Controls					
9 Subjects	39 ± 3	249 ± 44	1.0 ± 0.8	82.9 ± 6.7	16.0 ± 8.0
Thrombasthenia					
M.C.	30	159	1.1	65.7	0
M.M.	40	119	0.3	72.0	0

Values after adjustment of citrate concentration in plasma to 19.7 m*M*. Values shown are means ± S.D.

The procedure for studying the interaction of platelets with subendothelium was identical with that described previously[1-3]. Briefly, anticoagulated blood (final plasma citrate concentration 19.7 m*M*) was circulated through the perfusion chamber at an average flow rate of 160 ml/min for 10 min. Everted segments of rabbit aortas, previously denuded of endothelium[1], were mounted on the central rod of the chamber. After the chamber was rinsed with saline[2], the blood vessel segments were fixed and embedded in Epon[1].

Interaction of the platelets with the subendothelial surface was evaluated morphometrically in approximately 0.8 μm thick sections stained with toluidin blue and basic fuchsin[1]. 4 different categories of interaction were distinguished: a) naked: subendothelium devoid of platelets; b) contact: platelets present at the subendothelial surface, but not spread out on it; c) adhesion: platelets tightly adherent to the subendothelium and spread out on it; this may have the appearance either of a monolayer of platelets or an accumulation of several platelet layers. Included in (c) but evaluated as an additional parameter, masses of platelets (adhering aggregates) at least 5 μm in height were defined as thrombi (d).

Contact (b), adhesion (c) and thrombus formation (d) were determined for each of the 3 vessel segments exposed to a single blood sample and the means determined. The source of the specimens was unknown to those evaluating them.

Ultrathin sections were cut from selected areas of the exposed vessel segments and from platelet pellets which were obtained from platelet-rich plasma and fixed and embedded similarly. The sections were contrasted with uranyl acetate and lead citrate and examined with a Philips EM 300.

Results. The morphometric evaluation revealed that adhesion of thrombasthenic platelets to subendothelium was normal in M.M. and slightly reduced in M.C. based on a lower normal limit of 69.5% adhesion (mean of 9 controls less 2 S.D.) (Table). The low hematocrit of M.C.'s blood might have contributed to the slightly reduced value, since platelet adhesion depends on the red cell concentration and is virtually zero in their absence under the present experimental conditions[10], in addition the lower platelet counts in M.C. and M.M. would tend to give lower values of platelet adhesion.

Similar values of platelet contact in patients and controls indicate that the platelets of the 2 thrombasthenic subjects spread normally on subendothelium when vessel segments were exposed to their circulating blood. By contrast, platelet aggregation was virtually absent. As indicated in the Table, platelet thrombus formation (referred to as an accumulation of platelets 5 μm or more in height) was nil in the 2 patients, whereas this type of interaction (Figure 1c) occurred on 16% of the subendothelial surface when exposed to the blood of control subjects. The findings were even more dramatic than indicated by the results in the Table. In the 2 thrombasthenic patients, only a tightly adherent monlayer of spread platelets was seen and virtually no platelet-platelet interaction (aggregation) observed (Figures 1a and 1b).

Examination by electron microscopy of isolated platelets and of selected areas of exposed vessel segments revealed 2 additional findings: 1. thrombasthenic platelets spread out on subendothelium, but rarely overlapped each other (Figure 2). At the sites where overlap (platelet aggregation) was observed, numerous collagen fibrils were usually associated with the platelets. 2. In patient M.C., α-granules were virtually absent in platelets adhering to subendothelium, whereas these subcellular organelles were numerous in his isolated platelets. The α-granule to mitochondria ratio was high (8–11) in the isolated platelets and very low (0.1–0.2) in adhering platelets, indicating extrusion of granules during the process of adhesion of thrombasthenic platelets to subendothelium.

With the blood of patient M.C. only, numerous granulocytes were observed adhering to the platelet monolayer or to the subendothelium itself (Figure 1c). Electron microscopy revealed that most of the adherent granulocytes were associated with spread platelets (Figure 3). This has also been observed in rabbit blood vessels whose endothelium had previously (40 min or more) been removed in vivo[1], but has not been observed on subendothelium which had been exposed to anticoagulated blood in the perfusion chamber. At present we have no explanation for this finding.

Discussion. Morphometric evaluation, together with electron microscopic observations, establishes that thrombasthenic platelets adhere and spread normally on subendothelium. In contrast, platelet aggregation, i.e.

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formation of platelet aggregates adhering to the subendothelial surface – a phenomenon readily observed using blood of control subjects – was virtually absent in thrombasthenia. These findings indicate that platelet adhesion to a thrombogenic natural surface, such as subendothelium, and platelet aggregation involve basically different mechanisms.

The lack of platelet aggregation and thus the absence of platelet thrombus formation in the perfusion chamber corresponds well to the established failure of thrombasthenic platelets to aggregate in plasma in the presence of various aggregating agents[4, 11]. The disappearance of platelet granules other than mitochondria during the process of adhesion to subendothelium suggests that subendothelium induces the release of constituents from

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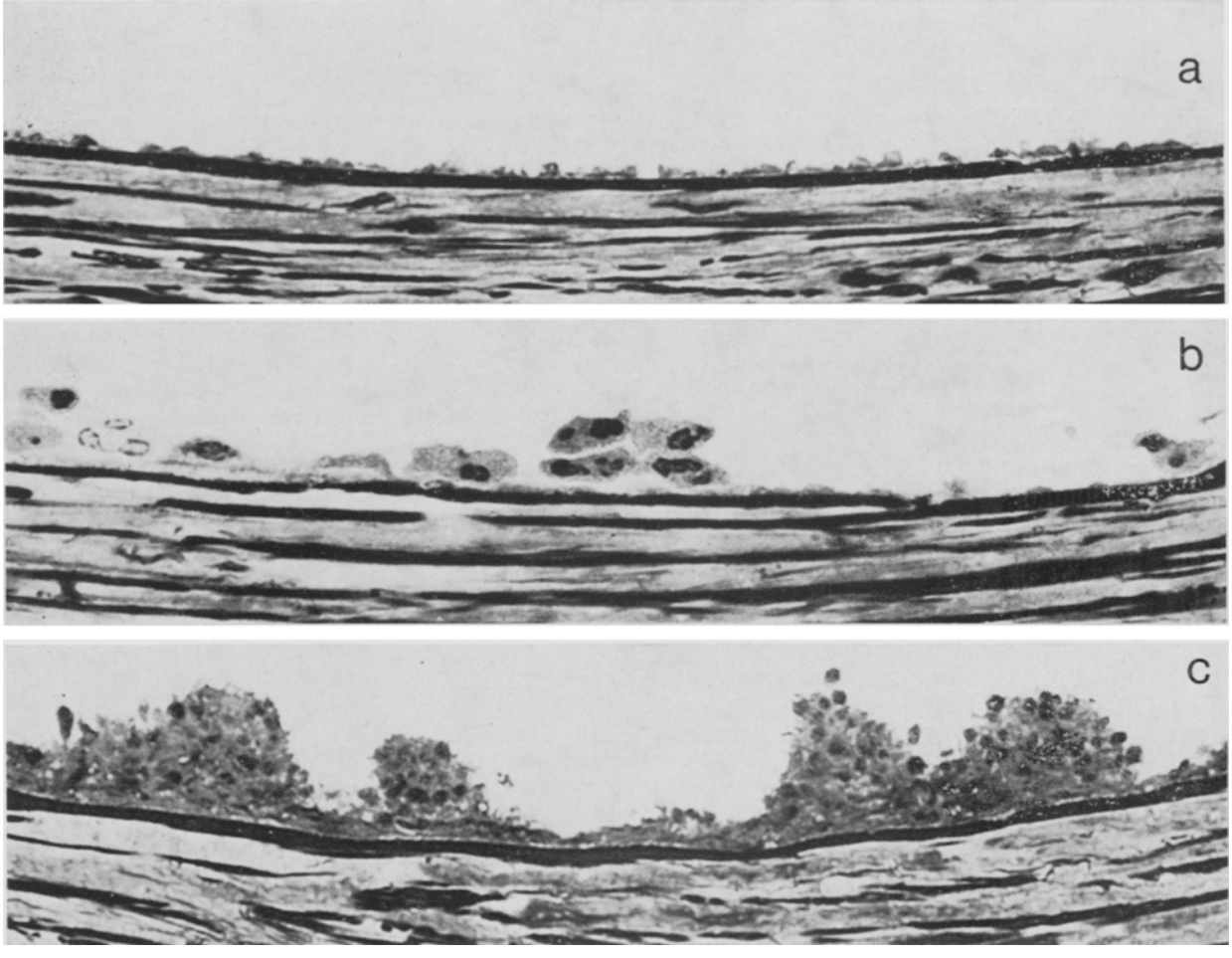

Fig. 1. Light micrographs of arterial subendothelium which had been exposed to anticoagulated blood for 10 min in the perfusion chamber. ×1300. a) Blood of a thrombasthenic patient (M.C.): platelet monolayer, no aggregation. b) Blood of a thrombasthenic patient (M.C.): leukocytes are seen adherent to the platelet monolayer and to each other. c) Blood of control subject: platelets adhere to the subendothelial surface and on top of each other (aggregation).

Fig. 2. Electron micrograph of thrombasthenic platelets adherent to subendothelium. The platelets are spread out but rarely overlap. The black bar indicates 1 μm.

thrombasthenic platelets. It has been previously demonstrated that the release reaction induced by collagen or thrombin in stirred platelet-rich plasma is normal in thrombasthenia[11]. In the present study, we examined the interaction of platelets in anticoagulated whole blood with a physiological surface (subendothelium) under conditions of blood flow that simulate those in arteries. Our findings demonstrate that the basic defect in thrombasthenia is neither in adhesion nor in the release reaction but rather in the mechanism involved in platelet aggregation. Exposure of subendothelium to flowing blood in the perfusion chamber has proved useful in further defining the nature of the platelet defects in several bleeding disorders. In VON WILLEBRAND'S disease[2] and in the BERNARD-SOULIER syndrome[3], we found decreased platelet adhesion, whereas aggregation was normal. In contrast, aggregation was markedly reduced in patients with storage pool disease or in normal subjects after aspirin ingestion[12], but never to the extent observed in patients M.C. and M.M. with classical thrombasthenia.

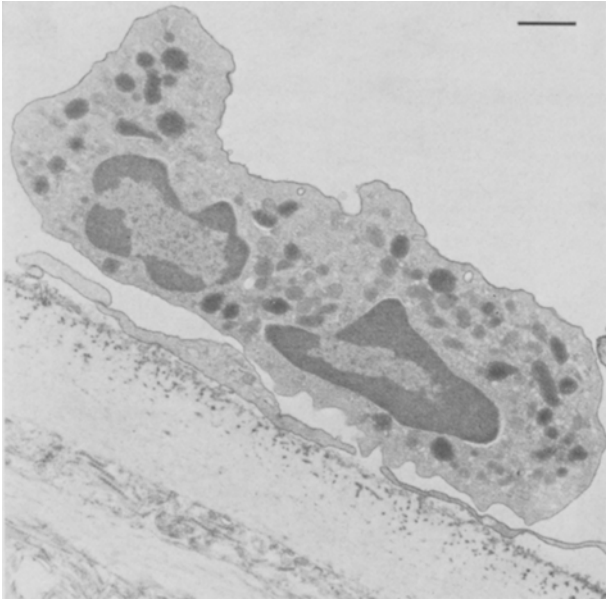

Fig. 3. Electron micrograph of a leukocyte in close association with thrombasthenic platelets spread out on the subendothelial surface. The black bar indicates 1 μm.

Zusammenfassung. Mit Hilfe einer neuen Perfusionskammer fand sich eine normale Adhäsion von Blutplättchen am Subendothel und eine fehlende Aggregation der Plättchen untereinander bei 2 Patienten mit Thrombasthenie GLANZMANN. Normale Ausbreitung der Plättchen am Subendothel zusammen mit einer normalen Degranulation und dem fehlenden Aneinanderhaften der Plättchen zum Aufbau eines Plättchenthrombus führen unter Berücksichtigung der an Patienten mit anderen Plättchenfunktionsstörungen gewonnenen Ergebnisse zum Schluss, dass Adhäsion und Aggregation auf verschiedenen Mechanismen beruhen.

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Fractions of Trapped Plasma in the Packed Red Blood Cells of Maternal, Fetal and Ox Bloods

Measurements of electrolyte and non-electrolyte concentrations of intraerythrocytes are occasionally made by using specimens of packed red blood cells separated from plasma by centrifugation. Small portions of plasma trapped in the specimens, the extents of which are dependent on the conditions of centrifugation[1], however, produce errors in the measurements of concentrations of intraerythrocytes. When more accurate measurements are required, results obtained from the packed red blood cells should be corrected for the volume of trapped plasma and concentrations in the plasma. Recently, we had an opportunity to take a measurement of chloride concentrations of plasma and intraerythrocytes in normal maternal, fetal and ox bloods in order to determine the Donnan distribution ratio of Cl^- between plasma and intraerythrocytes from each sample. In the course of chloride determinations of the intraerythrocytes, we needed results concerning the volume of trapped plasma for these 3 types of blood. However, since no such studies of these types of blood were found in the literature, the present experiment was designed to investigate this problem.

Materials and methods. Immediately after delivery, 5 ml each of maternal and fetal blood were sampled from the arm vein and the umbilical cord, respectively. Ox blood was sampled during venesection at a slaughterhouse. Heparin was used as an anticoagulant. The volume of trapped plasma was determined using basically the method of JACKSON and NUTT[1]. $^4/_{100}$ ml of 10% T-1824 dye-Ringer solution were added by microsyringe to 2 ml of the blood sample which had been placed in a larger syringe, and the 2 fluids were thoroughly mixed. Each of the samples of dyed blood thus prepared and undyed blood (original blood) was infused into a micro-hematocrit capillary (1.5 mm in inside diameter and 75 mm in length) and centrifuged at $10,200 \times g$ for 5 min. Red cell volume (hematocrit) was then measured excluding the buffy coat. Packed red blood cells were separated from the buffy coat and plasma by cutting the capillary 1 mm below the buffy coat, and an aliquot of 0.03 ml of it was pipetted into 2.0 ml of Ringer solution placed in a siliconized

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