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.

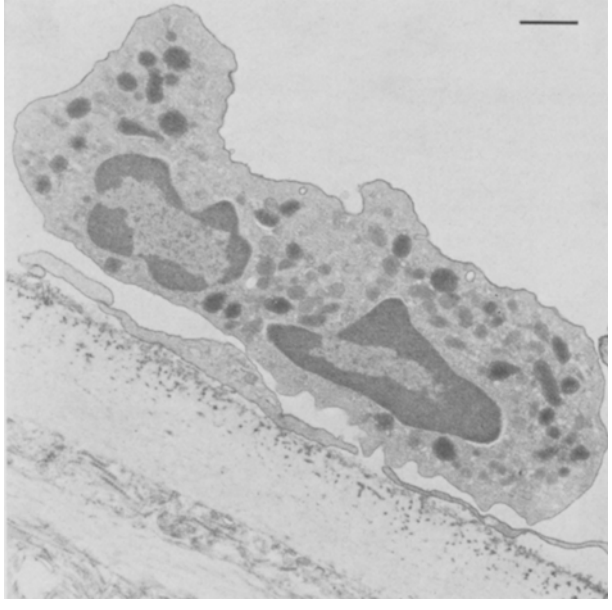

Fig. 3. Electron micrograph of a leukocyte in close association with thrombasthenic platelets spread out on the subendothelial surface. The black bar indicates 1 μm.

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

[1] D. M. JACKSON and M. E. NUTT, J. Physiol., Lond. *115*, 196 (1951).

centrifuge tube. The tube was gently rotated to mix both solutions thoroughly and centrifuged at 3,000 rpm for 10 min to obtain a clear supernatant. The optical density of the supernatant obtained from the dyed blood was read at 570 nm against the supernatant obtained from the undyed blood. Dyed plasma separated from the packed red blood cells was used for preparation of a standard solution of the dye. $^3/_{100}$ ml of the dyed plasma was pipetted into a 50 ml-graduated flask and diluted with Ringer solution. The optical density of the standard solution was read against Ringer solution. In the course of pipetting 0.03 ml of packed red blood cells into 2.0 ml of Ringer solution as mentioned above, the red blood cells adhering to the pipette were washed several times with the Ringer solution so that any visible trace of the red blood cells was transfused into the solution. This procedure was done very carefully in order to prevent hemolysis. If the supernatants were reddish, the results from these samples were discarded. Percent of plasma trapped in the packed red blood cells was calculated according to the method of JACKSON and NUTT[1]. Red blood cell counts were determined by a routine technique under a light microscope. Mean corpuscular volume (MCV) was calculated as red cell volume/red blood cell counts.

Results and discussion. The Table shows that, under our experimental conditions, the trapped plasma content in the packed red blood cells was 1.5% for maternal blood and 1.1% for fetal blood, the difference between these values being significant ($p < 0.02$ according to Student t-test). In ox blood, the percentage was markedly high, at 5.3%. The Table also indicates that the magnitude of trapped plasma is inversely related to MCV, i.e., the smaller the MCV, the higher the percentage of trapped plasma. This relationship supports the results of CHIEN et al.[2] in which percentages of trapped plasma were 1 to 3% in bloods of elephant, man and dog (MCV 112 to 72 μm^3), 4% in sheep blood (MCV 37 μm^3) and 9% in goat blood (MCV 18 μm^3) under the conditions of centrifugation at $15,000 \times g$ for 5 min. The MCV values for maternal, fetal and ox bloods obtained in our study were quite similar to those described elsewhere[3]. When erythrocytes of small diameters, such as those found in ruminants, are subjected to experiments such as hematocrit determination and concentration measurements using specimens of packed red blood cells, errors caused by intercellular plasma trapping should be considered in order to obtain more accurate results.

Percent of trapped plasma and mean corpuscular volume (MCV)

Blood	Trapped plasma (%)	MCV (μm^3)
Maternal	1.5 ± 0.10 (15)	96.5 ± 3.94 (6)
Fetal	1.1 ± 0.11 (13)	116.2 ± 5.16 (7)
Ox	5.3 ± 0.29 (15)	60.0 ± 3.34 (6)

The values represent mean ± SE. Numbers of samples measured are shown in parentheses.

Zusammenfassung. Blutproben verschiedener Herkunft enthielten nach 5 min Zentrifugation bei 15000 g im Erythrozytensediment folgenden Plasmagehalt: menschliches Erwachsenenblut 1,5%, menschliches Nabelschnurblut 1,1% und Ochsenblut 5,3%.

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Hyperacute Graft Rejection in *Eisenia foetida typica* and *Eisenia foetida unicolor*

The acceptance or rejection of grafted tissues and organs has been extensively studied in vertebrates and much knowledge has been gained of the underlying immunological mechanisms which lead to rejection of allografts and xenografts in immunologically competent recipients. In an attempt to study the evolutionary origins of these mechanisms, some preliminary investigations have been carried out in invertebrate systems, particularly in the earthworms where transplantations of body wall provides a convenient assay procedure.

Autografts, allografts and xenografts have been studied in *Eisenia foetida* and *Lumbricus terrestris* and the degree of rejection has been monitored by the loss of pigment from the graft surface[1-3]. Most autografts heal in successfully and are fully accepted; allografts are initially accepted but later undergo acute (2–20 days) or chronic (20–250 days) rejection; xenografts are usually rejected in an acute manner. Earthworms carrying a primary graft generally reject a second allograft or xenograft in an accelerated fashion similar to the 'second set rejection' of vertebrates and indicating immunological memory. It is also claimed[4] that accelerated rejection may be achieved in an unsensitized recipient by transferring coelomocytes from the putative donor.

In all the published reports there is mention of extremely rapid graft rejection occurring within 24 h. This rapid rejection is also seen in a proportion of autografts and has therefore been ascribed to 'technical failure' rather than incompatibility. From initial experiments, I gained the impression that this 'hyperacute' rejection was more often seen with allografts and xenografts than with autografts. The present investigation was designed to determine whether this was indeed so, and whether a proportion of graft rejections put down to technical failure might instead be related to incompatibility between donor and recipient.

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