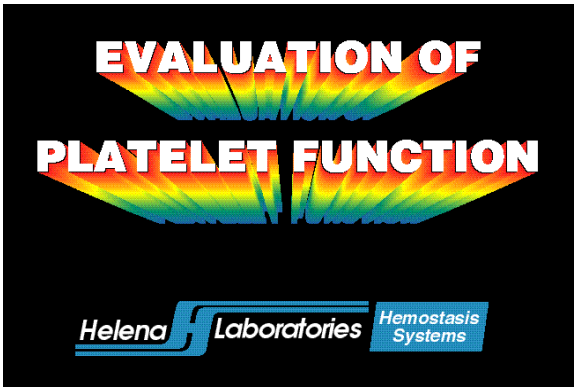
EVALUATION OF PLATELET FUNCTION

Cat. No. 5391

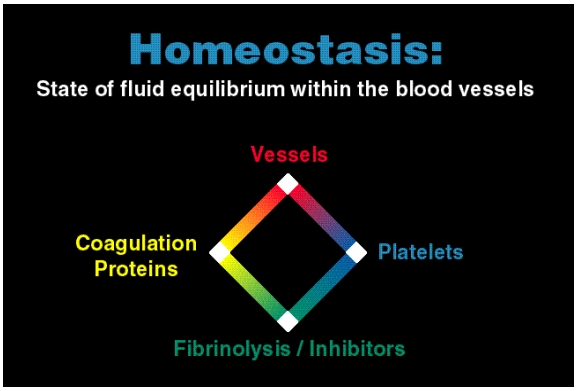
Helena Laboratories
Beaumont, Texas
Hemostasis Systems

Evaluation of Platelet Function



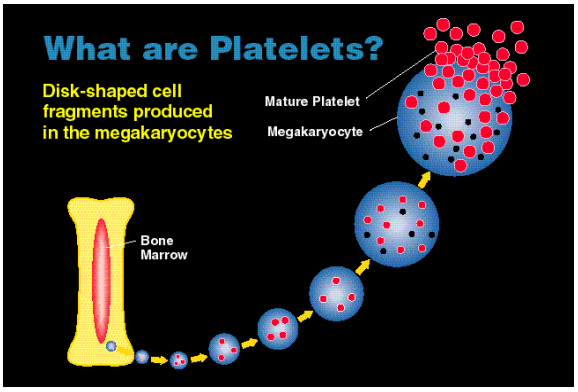
SLIDE 1
HELENA LABORATORIES is pleased to present this 35 mm slide series on the "Evaluation of Platelet Function".

This slide series briefly reviews what platelets are and their role in clot formation. A significant portion of this slide series describes platelet defects and tests that can be used to identify these disorders.

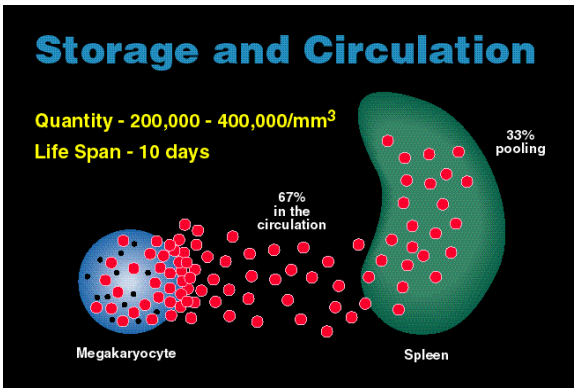


SLIDE 2
Homeostasis describes the normal condition of the circulatory system whereby fluid equilibrium is maintained within the blood vessels. This equilibrium is built on interactions between the vessels, coagulation proteins, fibrinolysis and inhibitors, as well as platelets. When the equilibrium is disrupted, it can result in bleeding or thrombosis.

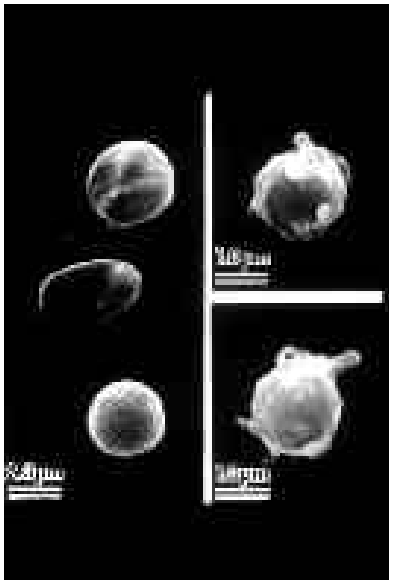
Note: Although most experts have expanded the term *hemostasis* ("arrest bleeding") to encompass what we are calling *homeostasis*, for the purpose of this slide series we will use the terms *homeostasis* and *hemostasis* in their more narrow context.



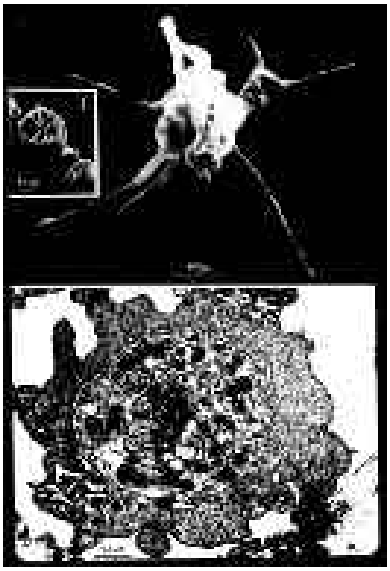
SLIDE 3
Platelets or thrombocytes are disc-shaped, anucleate cell fragments produced from megakaryocytes in the bone marrow. Megakaryocytes develop by a process of endomitosis or nuclear proliferation without cytoplasmic division. At the end of the eighth nuclear stage, cytoplasmic maturation begins and is characterized by the appearance of diffuse granulation and "demarcation membranes". These demarcation membranes represent formation of platelet plasma membranes. They eventually fuse, resulting in the "shedding" of cytoplasmic fragments of the megakaryocyte as platelets. The hormone thrombopoietin controls both megakaryocyte and platelet production.



SLIDE 4
After release from the bone marrow platelets circulate in the blood. In the normal individual 67% of the platelets are in general circulation with approximately 33% being pooled in the spleen. Platelet concentration is normally 200 to 400 thousand platelets per cubic millimeter of blood. Platelets may be counted by phase microscopy or electronic particle counting equipment. Newer technology and instrumentation now allow estimation of platelet size. Platelet sizing is thought to be a good indicator of platelet production and/or platelet destruction. In general, the younger more robust platelet, is larger than the older platelet. The normal life span of a platelet is 8 to 10 days.

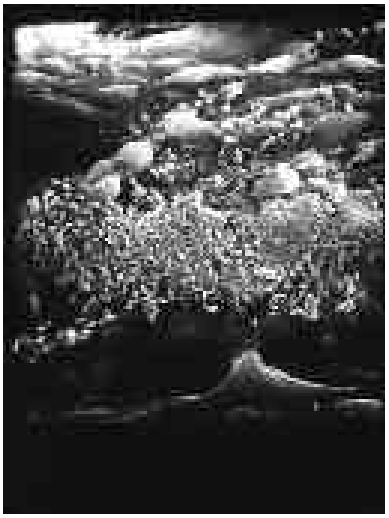


SLIDE 5
Pictured here are electron photomicrographs of platelets kindly provided by the late Dr. Marion Barnhart of the Department of Physiology, Wayne State School of Medicine, Detroit, Michigan. On the left side of the slide, we see three relatively normal disc-shaped platelets. On the right side of the slide, we can see the beginnings of pseudopod formation of "activated" platelets. As we will learn in a few minutes, this is an essential part of platelet function.

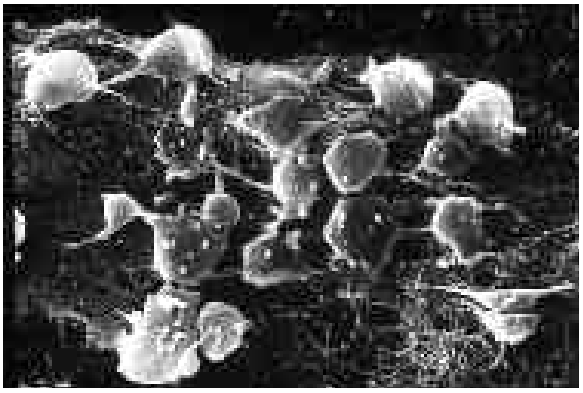


SLIDE 6
Here we see two types of electron photomicrographs. The scanning photomicrograph at the top of the slide shows an activated platelet with marked shape change and dramatic pseudopod formation. The inset photo shows a cross section of the platelet. With this dramatic view of the inside, we can see the surface connecting tubules.

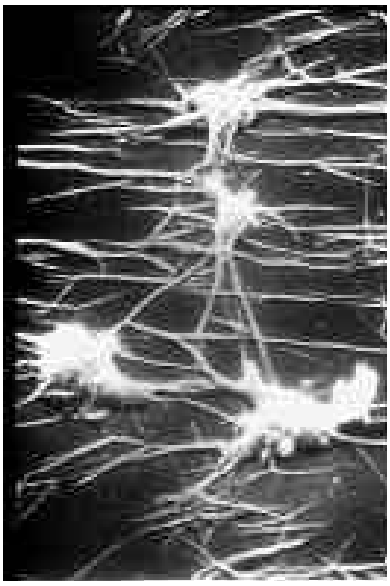
The lower portion of the slide shows a transmission photomicrograph. Notice the early pseudopod formation and the organelles being pushed to the side of the platelet. Platelets may be activated "in vivo" by response to vessel damage; usually an exposed collagen layer and/or a change in the electrical charge on the surface. When this occurs, platelets are called to the site of injury. Here they adhere to the foreign surface, change shape (viscous metamorphosis), release granular material (platelet release reaction), and stimulate additional platelets to continue the cycle.



SLIDE 7
Here we can see various platelet aggregates lining an area of exposed endothelium. This is an example of platelet adhesion and the beginning of the platelet response mechanism.

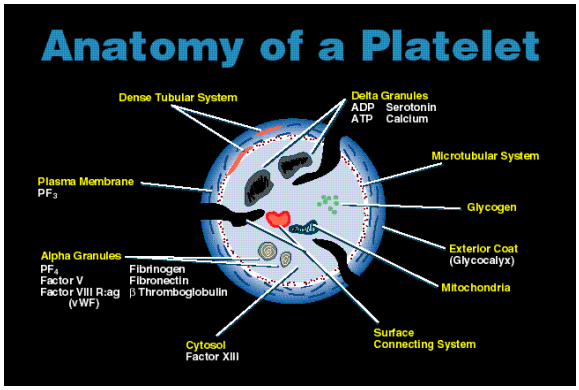


SLIDE 8
This slide lets us take a closer look at platelets adhering to a collagenous surface. Fibrin strands can also be seen. These strands are bound by the platelet glycoprotein IIb-IIIa receptors.

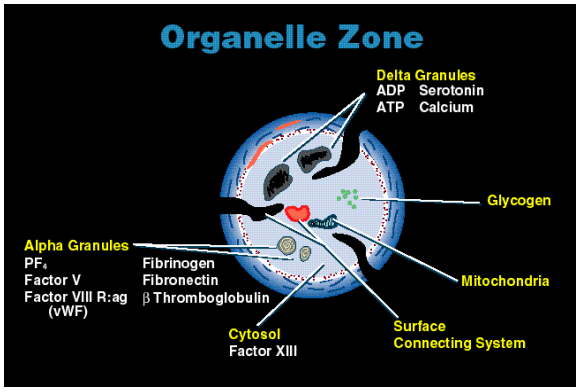


SLIDE 9
This slide shows platelets on a glass surface. Here we can see more clearly how they adhere to the surface and how they aggregate or stick to one another. The shape change and extensive pseudopodia extension results in an up regulation of the IIb-IIIa receptors for solidification of the fibrin clot during the contractile process.

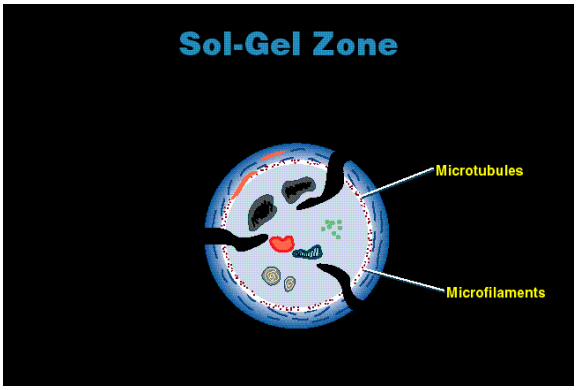
SLIDE 10
Non-viable and aged platelets and platelets which have fulfilled their hemostatic functions are cleared from circulation, primarily by the spleen and liver. This slide depicts the macrophage destruction of no longer needed platelets.



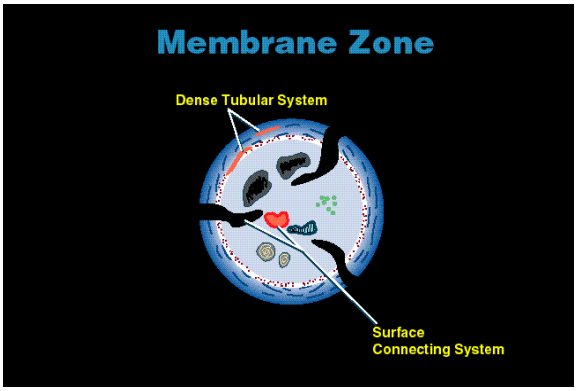
SLIDE 11
Here's a schematic representation of the structures within the platelet. In the next few slides we'll work our way from the inner most structures to the outside surface of the platelet. It should be noted here that the surface connecting system is found throughout the platelet. This series of channels enables the platelet to take up substances from the plasma, as well as release intraplatelet substances from the storage granules. It is through this system that many of the functions of the platelet are carried out, including secretion into the plasma of intraplatelet substances associated with the platelet release mechanism. While the channels penetrate the surface of the platelet in a random manner, inside the platelet the channels are generally located in close proximity to the granules and organelles.



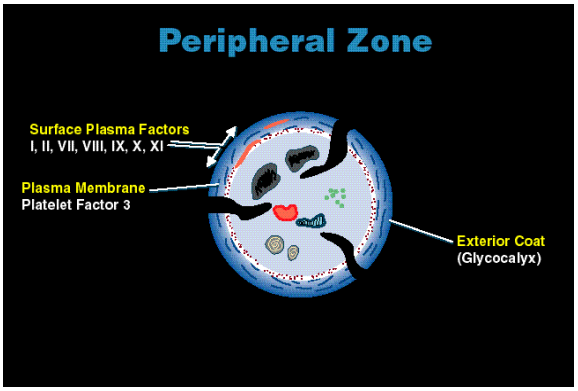
SLIDE 12
The organelle zone of the platelet contains alpha and delta granules, cytosol, glycogen for energy storage, mitochondria, as well as the surface connecting system. Of the two types of granules found in the platelet cytoplasm, the majority are of the alpha type. Alpha granules contain platelet factor 4, fibrinogen, fibronectin, factor V, von Willebrand factor and ß-thromboglobulin. The second type of granule is the delta (formerly called dense body), which is the storage site for ADP and ATP, serotonin and calcium. Lyso–somes, which contain enzymes such as cathespin and acid hydrolases, are also found in the organelle zone.



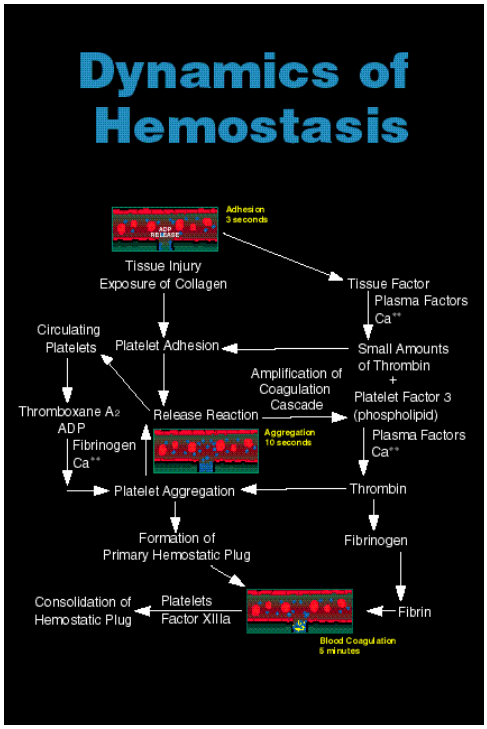
SLIDE 13
Moving outward from the organelle zone, we come to the sol-gel zone. This zone contains numerous submembrane microfilaments and microtubules. These microstructures form the platelet skeleton which maintains the circulating discoid shape and position of the organelles. There is also a secondary system of microfilaments which, during the contractile phase, maintains internal organization and secretion.



SLIDE 14
The membrane zone also contains the surface connecting system, as well as the dense tubular system. The dense tubular system appears to respond to calcium sequestration by initiating centralization of the internal organelles. Arachidonic acid metabolism is mediated in the dense tubular system.

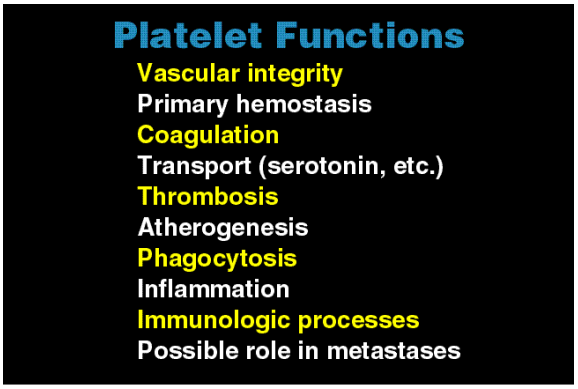


SLIDE 15
The exterior coat or glycocalyx is a phospholipid matrix sometimes described as the fluffy “plasmatic atmosphere”. The glycocalyx contains plasma proteins and carbohydrate molecules related to the complement, coagulation, and fibrinolytic systems. The peripheral surface of the platelet contains plasma factors I, II, VII, VIII, IX, X and XII. Platelet factor 3, a phospholipid involved in most of the intrinsic clotting mechanisms, is also contained in the plasma membrane. All of the glycoprotein binding sites (receptors) are located in this zone.



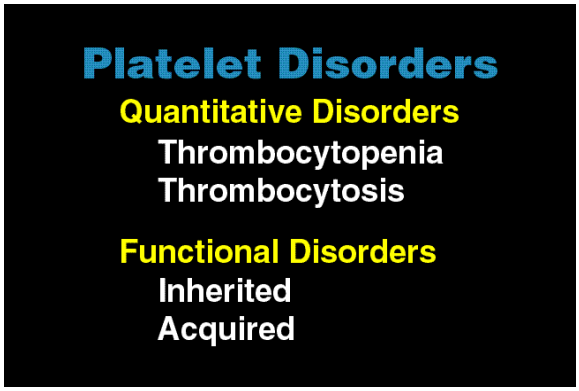
SLIDE 16

This shows the dynamics of the hemostatic mechanism. When injury to a tissue breaks the endothelial lining and exposes collagen or the basement membrane, platelets immediately begin to adhere to the exposed surface. The adhering platelets release ADP, epinephrine, and serotonin from their granules which initiates aggregation of more platelets. At the same time tissue thromboplastin is released from the surrounding tissue and mixes with the plasma factors and calcium to generate small amounts of thrombin. The thrombin enhances the platelet adhesion and aggregation. The coagulation cascade has also been activated with formation of a fibrin clot in about 5 minutes. As their final function, platelets, in the presence of factor XIII, consolidate and stabilize the fibrin plug.



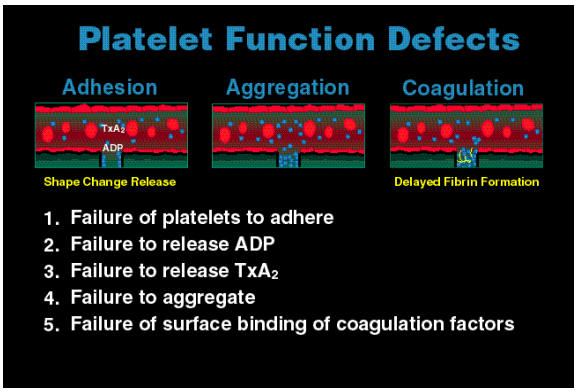
SLIDE 17

As the platelets mature and appear to die through the aging process, various portions of the platelets are removed and utilized in repair and support of the vascular system. The aged platelets are removed by the spleen and liver. The platelet is responsible for adhesion and primary hemostasis. Because the platelets also contain many of the plasma clotting factors they play a very important role in the coagulation response and formation of the fibrin clot. Platelets transport a number of materials such as serotonin. They are involved in thrombosis, atherogenesis, phagocytosis, inflammation and immunologic processes. A number of investigators have reported the possible role in metastatic diseases.



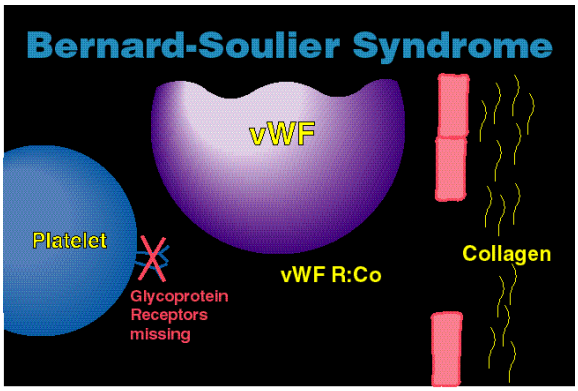
SLIDE 18

We generally classify platelet disorders as either an abnormality in *number* or *function*. Quantitative disorders such as thrombocythemia relate to too few platelets, while thrombocytosis relates to too many platelets. We can characterize defects in platelet function as inherited disorders and/or acquired disorders. While it is important to note that platelets must be normal in both *number* and *function* in order to maintain homeostasis, we will deal primarily with the functional aspects of platelets.



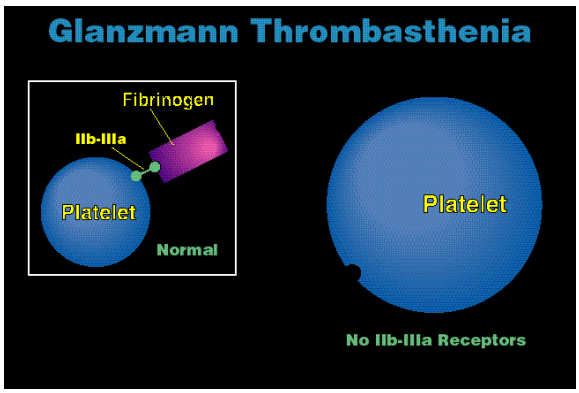
SLIDE 19

Inherited platelet function defects interfere with the platelet's ability to adhere to subendothelium, to release ADP or thromboxane A_2, to aggregate, or to bind with coagulation factors. Many of these same functions may also be affected as the result of acquired problems. Thromboxane A_2 and ADP release affect shape change. Delayed fibrin formation results from failure to bind to surface clotting factors. Among the inherited disorders affecting platelet function are von Willebrand disease, Bernard-Soulier syndrome, Glanzmann thrombasthenia, storage pool deficiencies and aspirin-like disorder.



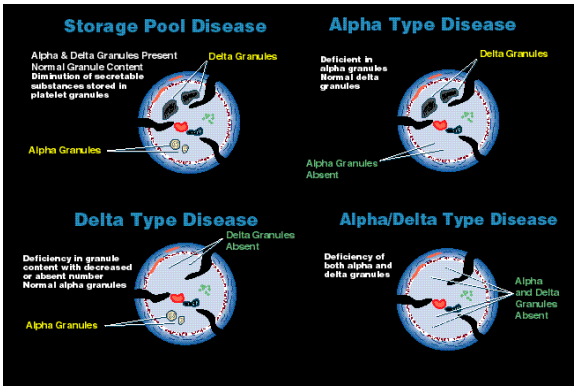
SLIDE 20

In Bernard-Soulier syndrome various glycoprotein receptors (Ib-IX complex and V) are missing from the surface of the platelet. The Ib-IX complex and V combines with the vWF/ristocetin cofactor portion of the factor VIII complex in order to complete the binding to surface collagen.



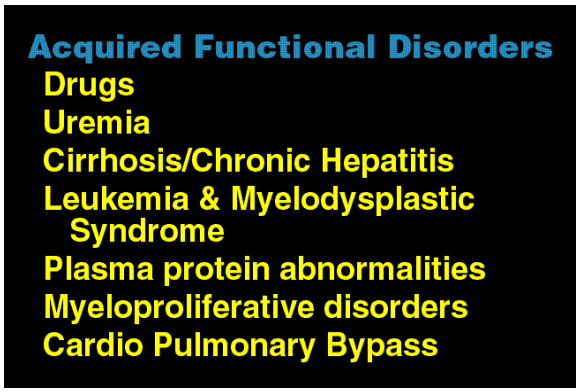
SLIDE 21

Glanzmann thrombasthenia results from a deficiency or abnormality in the IIb-IIIa complex of glycoprotein receptors. This is the binding site for fibrinogen as well as other plasma proteins.

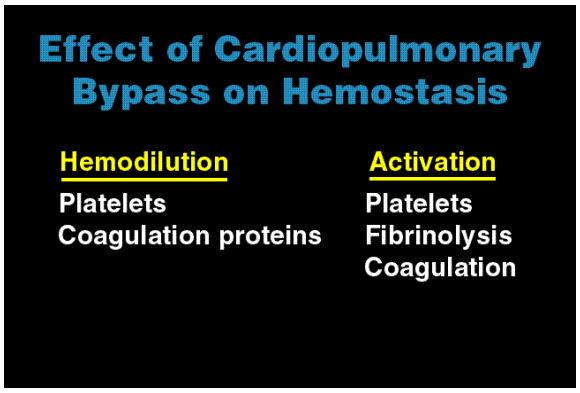


SLIDE 22

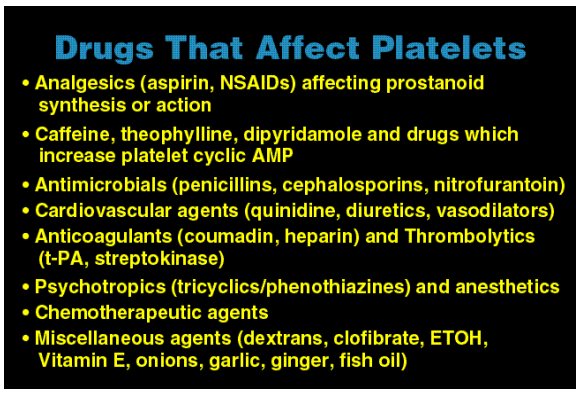
Storage pool deficiencies are characterized by decreased numbers of alpha and/or delta granules within the platelet. The content of the granules may be affected as well as the number of granules. Delta disease patients exhibit mild to moderate bleeding. Patients with alpha granule deficiencies usually have mild bleeding.



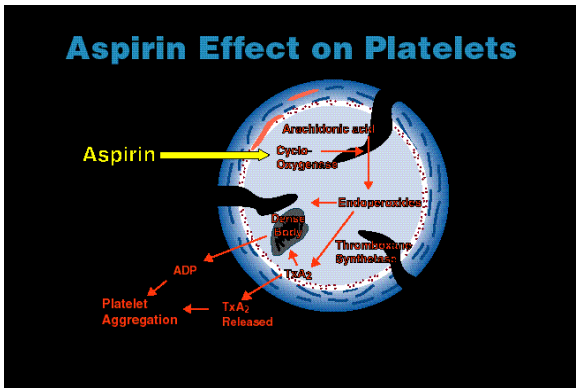
SLIDE 23
Functional platelet disorders can be acquired secondary to drug therapy, uremia, cirrhosis, leukemia, plasma protein abnormalities and myeloproliferative disorders. The majority of patients presenting with platelet problems today do so as a result of an acquired dysfunction.



SLIDE 24
In another type of acquired disorders, we see activation of platelets and disruption of homeostasis resulting when blood is removed from the body and recirculated. Such is the effect of cardiopulmonary bypass surgery on hemostasis. A number of things happen. One is hemodilution for both the platelets and the coagulation proteins. There is activation of platelets, the fibrinolytic system, and the coagulation system. The plasmin generated as a result causes alterations of many glycoprotein receptor sites, primarily IIb-IIIa, resulting in platelet dysfunction.

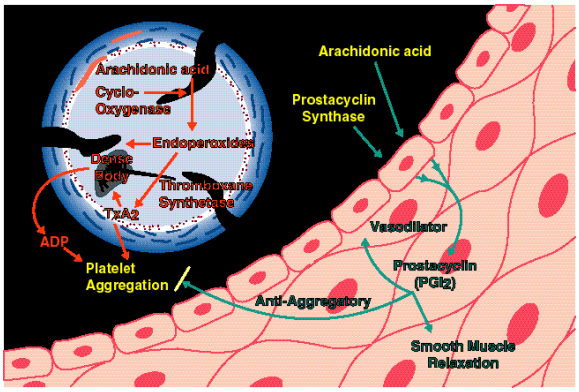


SLIDE 25
Drugs such as aspirin, NSAIDs (nonsteroidal anti-inflammatory drugs), dipyridamole, clofibrate, and dextrans can affect platelet function. Even agents as common as Vitamin E, caffeine, garlic and onions, can affect platelet function. Some effects such as those from sulfinpyrazone are reversible, where others such as aspirin are irreversible.



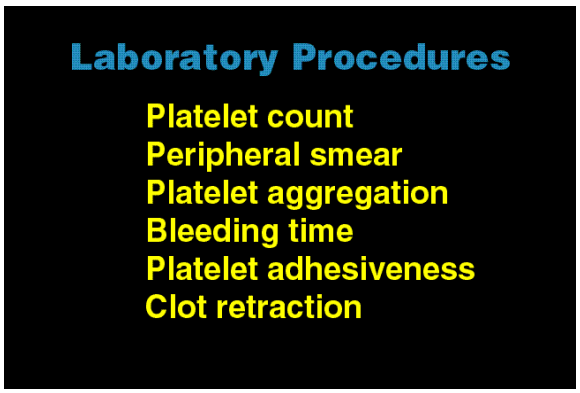
SLIDE 26

Aspirin causes acetylation of the enzyme cyclo-oxygenase. This irreversible reaction prevents conversion of arachidonic acid to endoperoxides. Thromboxane A_2 plays a role in the secretion of delta granule contents. The presence of aspirin impairs this sequence of events.



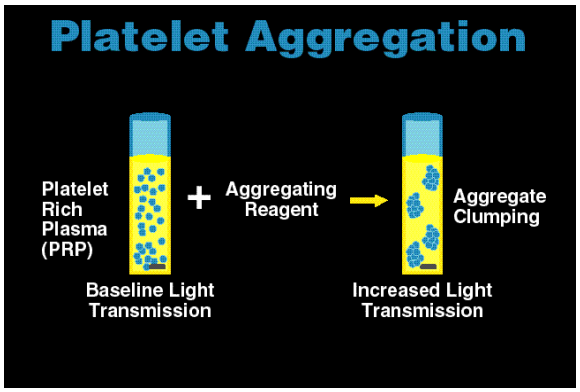
SLIDE 27

When an injury occurs and the platelets are activated, arachidonic acid is enzymatically released and converted into thromboxane A_2, a potent platelet agonist. Arachidonic acid is also metabolized in the endothelium to prostacyclin, a very potent inhibitor of platelet function. These two arachidonic acid cycles complement each other in maintaining a homeostatic balance. It should be noted that aspirin in low doses affects only the arachidonic acid metabolism in the platelets and not in the endothelial cells.



SLIDE 28

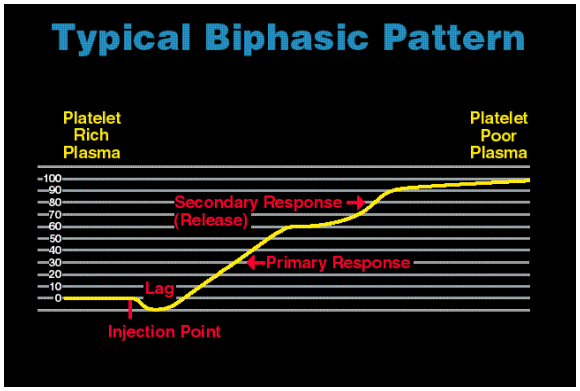
We will now examine some of the laboratory procedures available for evaluation of platelets. Platelets can be evaluated in terms of quantity and/or quality. As stated earlier, the normal range is approximately 200,000-400,000 platelets/mm^3 of blood. If the platelet count drops below 100,000 homeostasis is out of balance and the patient is predisposed to hemorrhage. Platelets must be normal in *number* and *function* in order to provide proper homeostasis. Therefore, a platelet testing profile should include platelet count, peripheral smear (looking for size and morphology), clot retraction, platelet aggregation studies, bleeding time and platelet adhesiveness studies.



SLIDE 29

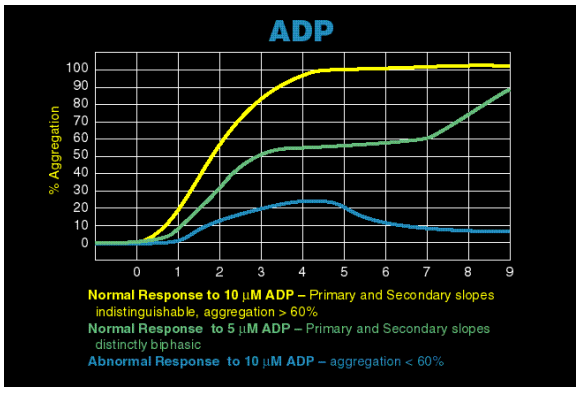
Platelet aggregometry studies are in-vitro assessments of platelet function. In principle, the test determines whether platelets will interact with other platelets to form platelet clumps when stimulated with various types of aggregating agents (agonists). Collagen, epinephrine, ADP, arachidonic acid, and ristocetin are commonly used agents.

The test begins by measuring the optical density of platelet-rich plasma (PRP). When an agonist is added to the platelet-rich plasma, the platelets clump, the plasma becomes less turbid, and light transmission through the plasma increases.



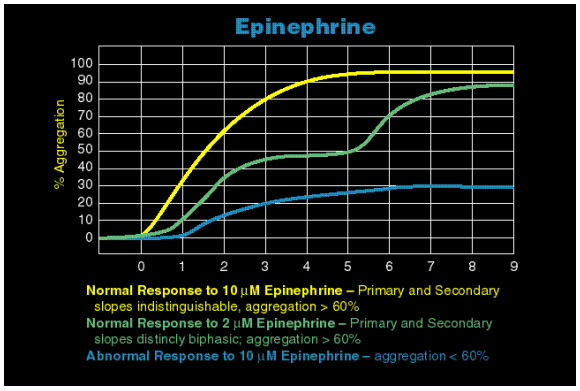
SLIDE 30

A typical biphasic aggregation curve is depicted here. Beginning at the bottom of the graph, baseline is established by passing a light beam through platelet-rich plasma (PRP) and setting the transmission at 0%. An agonist is added and the platelets begin to aggregate or form clumps, which allows more light to pass through the cuvette and the recorder shows an increase in light transmission. Direct aggregation of platelets by the aggregating agent is called “primary aggregation”. With some agonists, such as epinephrine, this is followed by a second wave of aggregation. This secondary phase results from release of ADP from the delta granules within the platelets (platelet release reaction), triggered by the initial aggregating agent.



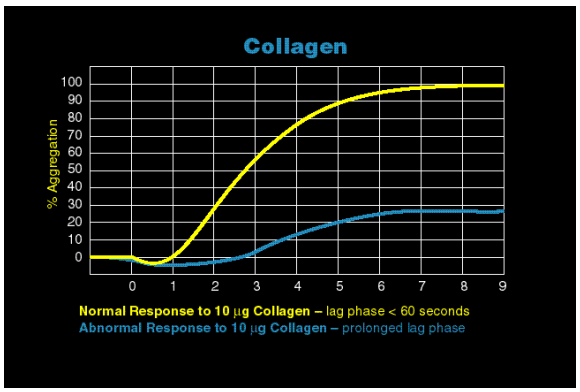
SLIDE 31

This is a representation of three types of responses with ADP. The yellow (top) line shows a normal primary response with an aggregation greater than 60% using 10 micromolar ADP. The green (middle) line shows a normal response with 5 micromolar ADP showing both the primary and secondary responses. The blue (bottom) line shows an abnormal response to 10 µm ADP with only a primary response of less than 60% aggregation.

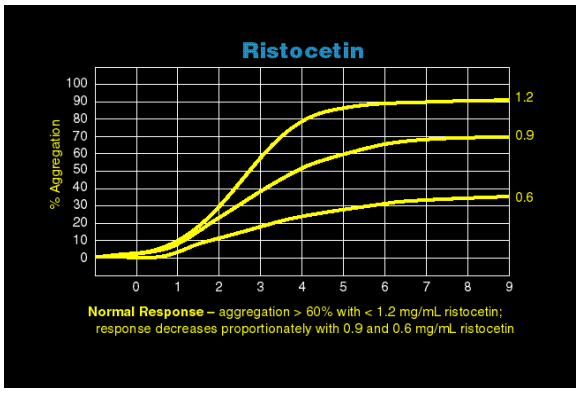


SLIDE 32

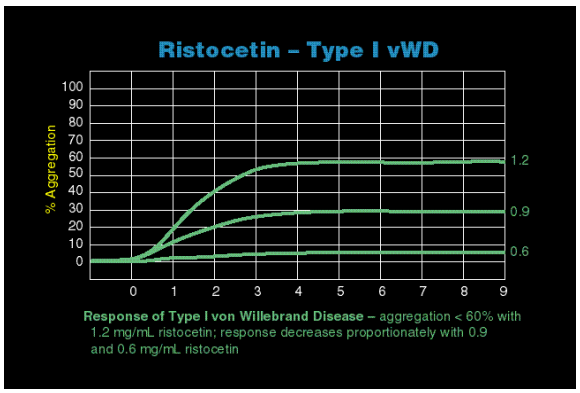
This is a representation of three types of responses with Epinephrine. The yellow (top) line shows a normal primary response with aggregation greater than 60% using 10 micromolar Epinephrine. The green (middle) line shows a normal primary and secondary response to dilute 2 micromolar Epinephrine. The blue (bottom) line shows an abnormal response to 10 micromolar Epinephrine with less than a 60% aggregation.



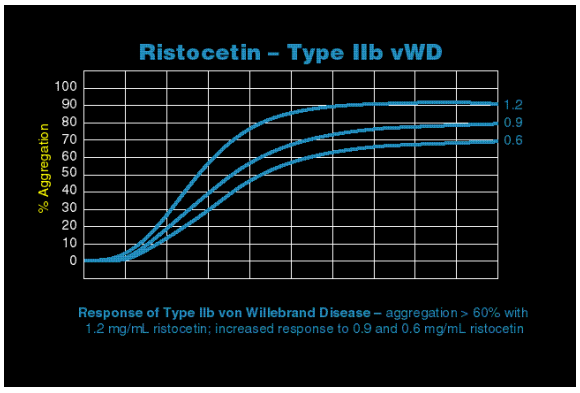
SLIDE 33
This is a representation of responses to 10 micrograms/mL equine collagen. The yellow (top) line represents a normal response with less than 60 seconds lag time and a >60% aggregation. The second response, shown in blue (bottom line), is an abnormal response to 10 micrograms/mL collagen with a long lag phase and <60% aggregation.



SLIDE 34
This is a representation of normal responses with Ristocetin. The top yellow line shows a normal primary response with an aggregation response greater than 60% using 1.2 mg/mL Ristocetin. The middle response line depicts a normal response with diluted Ristocetin (0.9 mg/mL), allowing a secondary response to be measured. The bottom line is a normal response to 0.6 mg/mL Ristocetin.



SLIDE 35
This shows typical platelet response in Type I von Willebrand Disease. With 1.2 mg/dL ristocetin, aggregation is less than 60%. Response decreases proportionately with 0.9 and 0.6 mg/dL ristocetin.



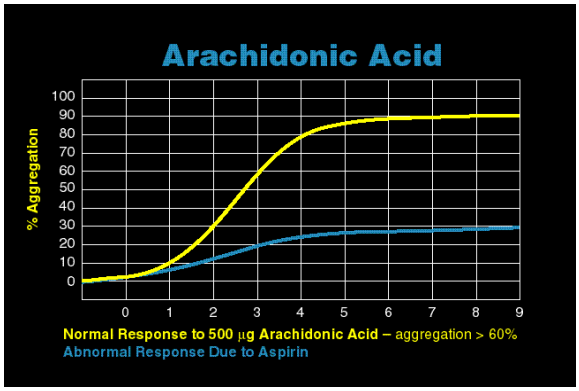
SLIDE 36
Platelet response in Type IIb von Willebrand Disease shows an abnormally high aggregation with low amounts of ristocetin (0.6 mg/mL or less). This is primarily caused by the presence of abnormal vWF which attaches to the platelets, creating a “sticky” platelet.

Interpretation of Factor VIII Testing

	Hemophilia A	Hemophilia A Carriers	Classic vWD (TYPE I)	Variant Type II d vWD	Variant Type II b vWD	Variant Type III vWD
Genetic Transmission	X-Linked	X-Linked	Autosomal Dominant	Autosomal Dominant	Autosomal Dominant	Autosomal Recessive
VIII:C	↓	50%	↓	Normal or slight ↓	Normal or slight ↓	↓
vWF:Ag	Normal or slight ↓	Normal	↓	Normal or slight ↓	Normal or slight ↓	Minute amounts or absent
vWF R:Co	Normal	Normal	↓	↓	Normal or slight ↓	Absent
Platelet Aggregation with Ristocetin	Normal	Normal	↓	↓	↑	Absent
Bleeding Time	Normal	Normal	Prolonged	Prolonged	Prolonged	Prolonged
Platelet Retention	Normal	Normal	↓	↓	↓	↓
Crossed Immuno-electro-phoresis	Normal	Normal	Normal migration rate	Abnormal arc	Abnormal arc	Variable (mostly abnormal)
Multimeric Structure	———	———	Normal in plasma and platelets	Absence of large and intermediate multimers from plasma and platelets	Absence of only larger multimers from plasma, normal in platelets	Variable

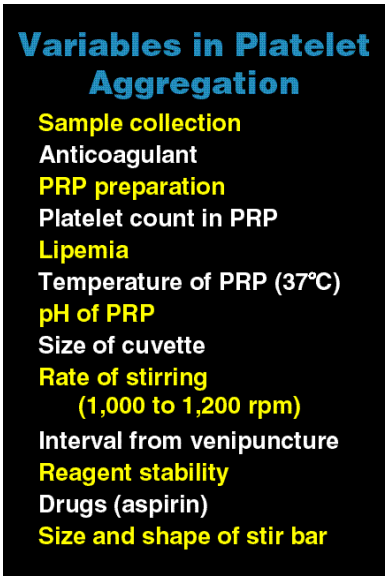
SLIDE 37

This slide describes the difference between hemophilia and vWD and the interaction of the plasma factors and platelet responses. The identification or classification of Factor VIII deficiency and von Willebrand Disease requires multiple laboratory tests. This slide shows the differences between hemophilia and the various types of von Willebrand Disease based on the interaction of plasma factors and platelet response. Some of these testing procedures are readily available for routine testing, while others need to be performed in specialized laboratories.

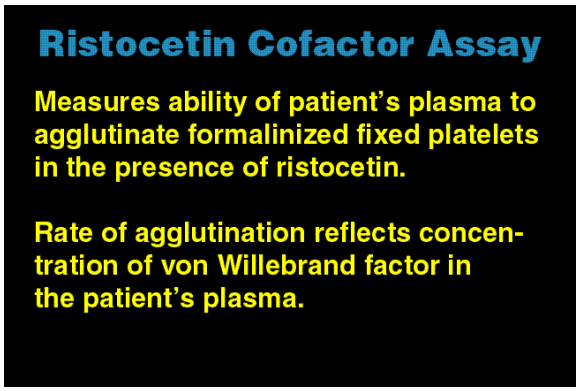


SLIDE 38

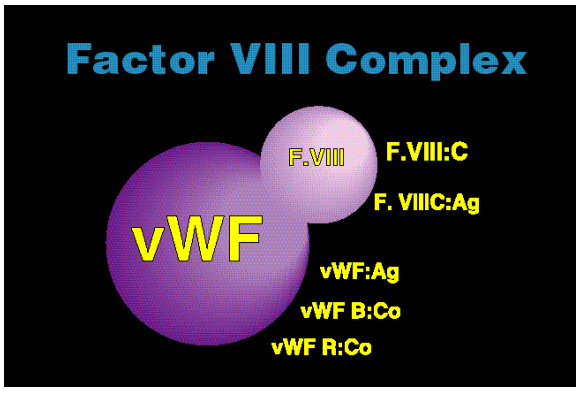
The yellow (top) line shows a typical pattern of response with 500 µg of arachidonic acid. The blue (bottom) line shows the response typically seen with aspirin intake.



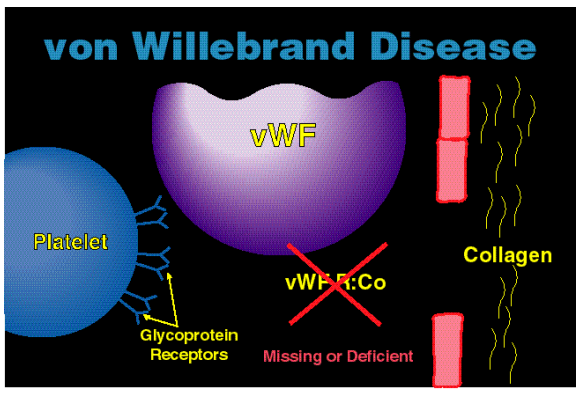
SLIDE 39
There are a number of variables in platelet aggregation. Factors affecting platelet aggregation include sample collection, the concentration of the anticoagulant, preparation of platelet rich plasma, the platelet count in the platelet rich plasma, lipemia, temperature of the platelet rich plasma, the pH of the platelet rich plasma, the size of the cuvettes, the rate of stirring, interval from venipuncture, stability of reagents, the effect of drugs, and the size and shape of stir bar.



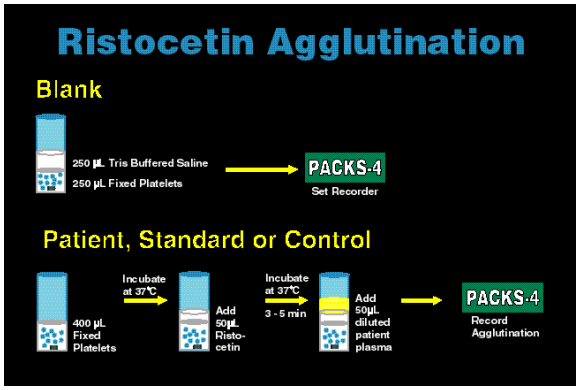
SLIDE 40
This is a description of the principles of the ristocetin cofactor procedure. The ristocetin cofactor activity assay measures the ability of a patient's plasma to agglutinate formalin-fixed platelets in the presence of ristocetin. The rate of ristocetin-induced aggregation is related to concentration of the von Willebrand factor in the plasma that is added. And the percent normal activity can be obtained from the aggregometer tracing.



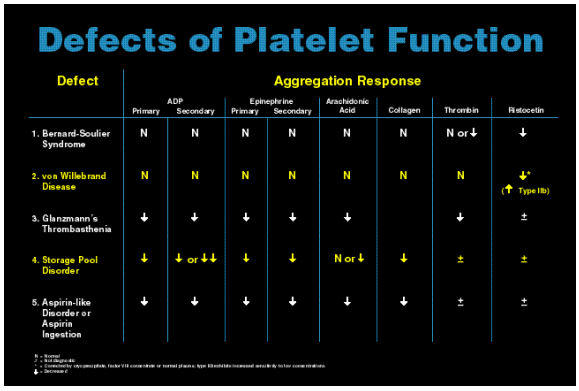
SLIDE 41
This is an artistic depiction of the Factor VIII complex. Although Factor VIII and von Willebrand Factor (vWF) are distinct proteins, they are closely related biologically. Factor VIII plays a role in the activation of Factor X. vWF is necessary for platelet adhesion. vWF is the larger molecular weight glycoprotein and appears to carry and stabilize Factor VIII in circulation. Ristocetin cofactor is the portion of the molecule measured by the ability of the patient's plasma to agglutinate lyophilized platelets in the presence of ristocetin and reflects an aspect of vWF.



SLIDE 42
von Willebrand Disease is characterized by missing or deficient vWF/ristocetin cofactor portions of the factor VIII complex. This results in the inability of this plasma protein to respond with the proper glycoprotein receptors (Ib-V-IX complex) and bind to the surface collagen.



SLIDE 43
This is a sample of a normal ristocetin agglutination procedure. In the ristocetin agglutination procedure, we prepare a blank solution of formalinized platelets which have a standardized count. Four hundred microliters of the blank are placed in the cuvette and added to the stir bar. The blank reading is then taken. Next 50 microliters of ristocetin are added to the platelets and allowed to equilibrate for 3 minutes. Then appropriate dilutions of the patient samples are added. Agglutination is recorded and calculated against the standard curve. The slope is proportional to the % Ristocetin Cofactor (vWF) present.



SLIDE 44
This chart compares the aggregation response seen in the various disorders affecting platelet function.

SLIDE 45
This is Helena's PACKS-4, **P**latelet **A**ggregation **C**hromogenic **K**inetic **S**ystem-**4**. It is a full-function 4-channel analyzer that allows platelet aggregation testing to easily and cost-effectively be performed as a routine part of any laboratory.

For more information on Helena's Platelet Aggregation System, call toll free 800-231-5663.

Helena Laboratories
Beaumont, Texas

Hemostasis Systems